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[*Authors alone are responsible for all opinions expressed in their Communications.*]

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No. 13.

ON ALGINIC ACID: ITS MODE OF OCCURRENCE AND ITS CONSTITUTION.

By THOMAS DILLON, M.A., D.Sc.,

AND

ANNIE McGUINNESS, M.Sc.,

University College, Galway.

[Read June 16. Published separately November 19, 1931.]

In 1883 Stanford (1), by extracting seaweed with sodium carbonate, obtained a viscid solution which, on acidulation, gave a gelatinous precipitate. To this gelatinous body he gave the name alginic acid or algin. Stanford's alginic acid was not a pure substance. Although he dialysed it, he never obtained it free from inorganic constituents. He believed it to be a nitrogenous compound. Subsequently nitrogen-free alginic acid was obtained by Krefting (2), by Kylin (3), and by Hoagland and Lieb (4). The paper of the latter authors contains a summary of the work done on alginic acid up to 1915.

In 1926 Atsuki and Tomoda (5) suggested that the acidic nucleus in algin was glucuronic acid, and, in the same year, Schmidt and Vocke (6) hydrolysed alginic acid from *Fucus serratus*, and claimed to obtain as a product glucuronic acid in the form of a crystalline cinchonine salt. In 1929 Nelson and Cretcher (7), working with *Macrocystis pyrifera*, concluded that purified alginic acid was "a polymer of the anhydride of an aldehyde sugar acid, in which all the aldehyde groups are conjugated and all the acid groups are free." They showed that the sugar acid was not glucuronic, but mannuronic acid. Recently they have isolated the lactone of mannuronic acid in a crystalline form from the products of hydrolysis. This appears to be the first record of the occurrence of mannuronic acid in nature. In a paper just published, Bird and Haas (8), working with laminaria, confirm the observations of Nelson and Cretcher.

Our work on this subject was begun in 1928, and concluded just before the appearance of Nelson and Cretcher's paper in June, 1929. The latter authors have not only confirmed the view that alginic acid is a polyuronic acid, but they have also settled the question of the configuration of the polymerising unit. The experiments we wish to record, while incidentally confirming the uronic acid theory, deal with two other aspects of the subject. One set of observations has a bearing on the problem of the mode of occurrence of alginic acid in the plant, while the other concerns the manner in which the uronic acid units are linked together to form the polymer.

The Mode of Occurrence of Alginic Acid.

Kylin suggests that alginic acid occurs in the cell wall in the form of an insoluble calcium salt, to which he gives the name algin. In the paper referred to above Bird and Haas show that the percentage of calcium in the plant is not sufficient for this theory. By exhaustive extraction with sodium carbonate, they found that Laminaria contained 60 per cent. of alginic acid, while the calcium content of the whole weed was found to be only 1·99 per cent. They conclude that "the cell wall of Laminaria contains alginic acid in two forms, one a soluble calcium magnesium alkali metal salt, present only to the extent of about one per cent., and the other in the free state, which appears to be the matter extracted by cold dilute sodium carbonate."

That free alginic acid should occur in the plant to the extent of 59 per cent., or anything approaching this figure, seems to us unlikely. Alginic acid is a relatively strong acid. It has an acid taste, and is acid to litmus and to methyl red. Laminaria, on the other hand, has not an acid taste, and the aqueous extract is not acid to litmus nor to methyl red. Moreover, free alginic acid gives a vigorous effervescence with sodium carbonate; whereas during the extraction of seaweed no effervescence is to be observed.

In order to gain exact information on this latter point, about ten grams of air-dried Laminaria fronds, cut into small pieces, were introduced into a flask, fitted with a one-hole rubber stopper, connected to a U-tube containing lime water, and having a guard tube full of soda-lime attached to its other limb. The weed was covered with sodium carbonate, and left standing. No bubbles were seen to pass through the lime water even when, after standing for more than an hour, the flask was vigorously shaken; nor was any milkiness observable. After fourteen hours the flask, the contents of which were now a gelatinous mass, was heated on a boiling water bath. The air which bubbled through the lime water produced no turbidity, even when the heating was continued for an hour.

One can scarcely attribute this complete absence of carbon dioxide evolution to adsorption on the colloid, as in that case some gas should come off on heating and shaking. We are therefore forced to the conclusion that the alginic acid in the plant is combined with a base sufficiently strong to form a stable carbonate.

The compound of alginic acid occurring in the plant will be by no means easy to isolate; but some experiments of ours appear to furnish a slight clue as to its character. The experiments were undertaken with a view to finding out whether any difference existed between the algin from the living weed and that from the desiccated plant. With this object, some fresh fronds of Laminaria were collected on the seashore. These were passed through a mincing machine, and the macerated product was covered with 5 per cent. sodium carbonate in a beaker and left standing for 24 hours. At the end of this period the beaker was heated for an hour on the water bath, and the contents were then filtered through flannel bags. From the viscid liquid the algin was precipitated by

hydrochloric acid as a light brown jelly. Following Hoagland and Lieb's process for the preparation of pure alginic acid, the jelly was redissolved in sodium carbonate, filtered through ordinary filter paper and reprecipitated. This was repeated three times. The sodium alginate was then precipitated by alcohol as a white "stringy" mass. This precipitate was dissolved in water, and was dialysed in parchment filters for three days, distilled water being used. Hydrochloric acid was now carefully mixed with the solution on the filter, and dialysis of the precipitated acid was continued for three days with tap water, and finally for four days with distilled water.

Hoagland and Lieb record that by the above process they obtained a specimen of alginic acid free from all but traces of ash. We, on the contrary, found that at the end of the treatment our dried product contained 3·7 per cent. of ash, which was increased by further dialysis. The jelly was, therefore, removed to a filter, and washed slowly with four litres of distilled water. The ash content was now 7·9 per cent. The acid was redissolved in sodium carbonate, the sodium alginate was precipitated by alcohol, dissolved in water, and put through the same process of dialysis of the sodium salt, and, finally, of the free acid, as before. The ash content rose to 10 per cent., at which value it remained practically constant. The ash had a light brown colour, and gave the flame test for sodium and calcium. It was insoluble in water, but soluble in dilute hydrochloric acid, giving a green solution, which was slightly cloudy. This solution gave a blood-red colour with potassium thiocyanate.

The above experiment was repeated with another sample of freshly-gathered Laminaria. During the final dialysis of the free acid daily determinations of the ash were made. Having fallen to a minimum of 3·03 per cent., it rose to 9 per cent.

We next proceeded to experiment with air-dried Laminaria fronds. When these were treated in exactly the same manner as the fresh material, we obtained without difficulty specimens of alginic acid containing from 0·6 to 0·7 per cent. of ash.

When the alginic acid is precipitated on the parchment filter there are evidently present some compounds containing calcium and iron, which will not pass through the filter. There is also an organic body which passes through much more slowly than the sodium chloride and the hydrochloric acid. When, therefore, the latter have all passed through the ash is at a minimum, and as the slowly dialysing organic matter continues to disappear from the filter the percentage of ash rises.

It would seem that in the growing plant the alginic acid is combined with colloidal compounds of calcium and iron, and that desiccation either destroys the colloidal character of these compounds or renders them insoluble, so that they are removed when sodium alginate prepared from dried fronds is filtered.

The Constitution of Alginic Acid.

Nelson and Cretcher by determinations of carbon and hydrogen obtained the formula $(C_6H_8O_6)_n$ for alginic acid. By titrating a weighed quantity of

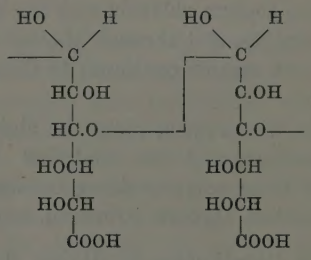
the dried substance with caustic soda they obtained a neutralisation value of 179, which agrees well with the figure (176) required by the above formula. From these results they arrive at the conclusion quoted above, that the polymerising unit is a sugar anhydride. Schmidt and Vocke, however, by titrating the dried acid found a neutralisation value of 184·8, which is by no means in such good agreement with the anhydride formula.

Carbon and hydrogen determinations on our purified and dried alginic acid gave a formula $C_6H_8O_6$, in agreement with Nelson and Cretcher. Our neutralisation value was obtained in a different way, and gave a different result. Instead of weighing and titrating dried alginic acid a quantity of the jelly was titrated, and the whole of the resulting sodium alginate solution was evaporated to dryness and heated to 100° C. until the weight was constant. Thus: 1·24 c.c. of normal caustic soda were required to neutralise a quantity of the jelly. The weight of sodium alginate obtained was 0·2728 g., corresponding to a neutralisation value of 198. $C_6H_{10}O_7$ would have a neutralisation value of 194.

We also determined the molecular weight of the acid by analysis of the barium salt. To this end a quantity of pure alginic acid jelly was neutralised with standard caustic soda, and a quantity of barium chloride equivalent to the soda used was added to the resulting solution of sodium alginate. Barium alginate came down as a fibrous, easily filterable solid, and was filtered off, washed and dried at 100° C. to constant weight. 0·1287 g. of this barium salt gave 0·0484 g. of barium carbonate. This corresponds to a molecular weight for the monobasic acid unit of 195·3.

The only interpretation of these results is that the dry alginic acid $(C_6H_8O_6)_n$ is a lactone. This lactone formation would account for the change in properties undergone by the substance when it dries.

If the dry alginic acid is a lactone then the polymerising unit is not a sugar anhydride analogous to cellulose and starch, but the complete sugar acid. Polymerisation could not then take place if the uronic acid unit existed in the ring form. It could only happen by the transfer of a hydrogen atom of an OH group of one unit to the aldehyde oxygen of another, giving the following formula:—



We have to thank the Ministry for Education for a grant, which enabled one of us to engage in this research.

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No. 14.

THE PERFORMANCE OF A RESERVOIR SUBJECTED TO FLOOD.

By H. H. JEFFCOTT.

[Read November 24. Published separately December 22, 1931.]

In the design of a reservoir it is essential that the spillway shall be of sufficient proportions to discharge excess water due to floods without causing an undue rise in the level of the water in the reservoir so as to endanger the dam. In deciding upon what this maximum level should be, allowance must be made for waves due to the action of wind. The height of waves on the reservoir surface depends on the intensity of the wind and on their "fetch," i.e. on the length of surface exposed to the wind and thus on the length of the reservoir.

For purposes of calculation it will probably suffice to take the height of wave in feet = $\cdot 05 L^{\frac{1}{2}}$ where L is the length of the reservoir in feet.

The type of spillway has next to be settled, i.e. whether of ordinary open type or a siphon. Usually the former type is adopted, and then its tentative dimensions have to be chosen, i.e. the width of spillway and its sill level. The latter figure determines the effective content of the reservoir, and so must be maintained as high as is compatible with a reasonable width and ample flood-discharging capacity. It will be convenient to measure the height h of the water surface of the reservoir above the sill level as datum, so that for such height h feet the discharge rate of the spillway is represented by Q cubic feet per second where Q is a known function of h.

For a rectangular weir Q is approximately $ah^{\frac{3}{2}}$, where a is a constant depending on its effective width in feet. For a siphon spillway Q is approximately $bh^{\frac{1}{2}}$, where b is a constant depending on its dimensions.

We have next to settle the magnitude of the flood input F cusecs to the reservoir and how it varies with time.

This depends on the intensity, spread, and duration of heavy rains in the catchment, on the manner in which the various tributary streams converge on the reservoir, on the presence of any lakes in the catchment, and how they are controlled in regard to discharge.

For the present purpose we presume we are given the curve of maximum flood discharge into the reservoir plotted against time, or an equivalent tabular statement.

Thus we suppose that the value of F corresponding to any time t is given. In

general it will not be possible to represent the relationship of F to t by a simple mathematical expression, and it is therefore best to develop the solution of the problem from a plotted or tabular knowledge of F.

Now during the period of flood inflow to the reservoir a certain utilised demand G cusecs will be drawn off, its magnitude depending on the duty of the reservoir. We presume that G is known in terms of the time t by a plotted curve or a table. Accordingly the net input P cusecs to the reservoir is $F - G$ at any time t. Thus we construct a curve or table for P.

As a refinement the curve for P should also be corrected by the addition of the rainfall (less evaporation) that falls on the reservoir's surface during the time considered, and by the addition of underground percolation into it, less leakage therefrom (if any).

Usually the area of the water surface of the reservoir increases with increase of height above datum. We require a curve or table giving the area A in square feet corresponding to a height h feet above the spillway sill. We assume such curve or table has been prepared.

We can now proceed to determine the performance of the reservoir.

Taking units of feet and seconds we suppose that at any time t seconds the height of water above sill datum is h feet. In a very short interval of time dt a net inflow to the reservoir of Pdt cub. ft. takes place.

During the same very small interval of time the height of water surface has increased by a very small amount dh, so that the quantity Adh cub. ft. is stored. Also the spillway, discharging at the rate Q cusecs, gets rid of a quantity Qdt cub. ft. in the small interval of time dt.

But the net inflow is equal to the quantity stored plus that discharged.

Hence $Adh + Qdt = Pdt$.

This equation can be solved for many special forms of A and Q as functions of h, and P as a function of t, but in general it is more convenient to proceed by a graphical or by a tabular method of solution.

Let t_1 and t_2 be two values of the time corresponding to which the heights of water surface are h_1 and h_2 respectively. Integrating between these limits we have

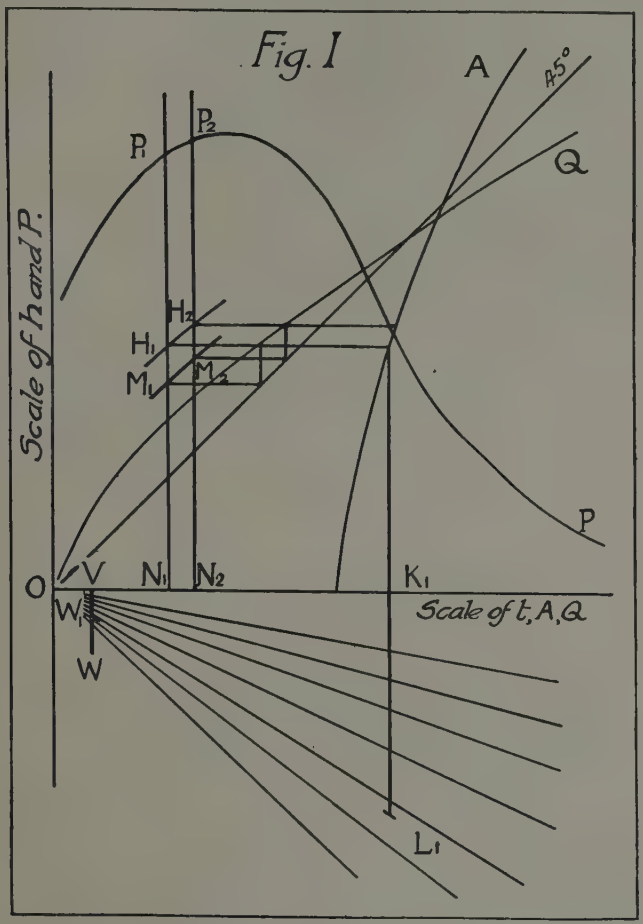
$$\int_{h_1}^{h_2} Adh = \int_{t_1}^{t_2} (P - Q)dt.$$

This equation is the basis of the graphical and tabular processes to be described. A similar method of solution has already been proposed by the author in other applications. (See Min. Proc. Inst. C.E.I., 1922, p. 157, and Sci. Proc. Roy. Dub. Soc., 1924, **18**, pp. 71-76.)

We take first the graphical solution. On squared paper we draw axes of ordinates and abscissae to represent h and t, respectively, to convenient scales.

Also to a convenient scale of ordinates we plot a curve for P against the abscissae t. (See fig. 1.)

We next plot a curve having as abscissae the values of A against h as ordinates, and also a curve plotted against h as ordinates having as abscissae the rate of discharge Q corresponding to assumed dimensions of the spillway.



Further, we draw a straight line through the origin at 45° to the axes, and below the axes of abscissae we draw a number of oblique straight lines as shown, all passing through the origin.

The various scales are to be so chosen that the diagram fits conveniently on the squared paper.

If 1 division or unit of length on the paper represents k feet (for h), l cusecs

of discharge (for P and Q), m sq. feet of reservoir area (for A) and n seconds of time (for t), then we require to make OV (see figure) equal to $\frac{ln}{km}(t_2 - t_1)$ divisions, where $t_2 - t_1$ is the time interval chosen for the operation expressed in divisions.

The diagram is now ready for use, and the various trial constructions may be conveniently made on tracing paper laid over it.

Choose a convenient small value for the interval $t_2 - t_1$. The accuracy of the solution and also the time required for it depend on the smallness of this interval. At the outset the inflow gradually fills the reservoir up to sill level. We choose the time to begin when the water is at sill level, and we proceed forward from this position according to the following method :—

Suppose the point $h = h_1 = N_1H_1$ corresponding to the time $t = t_1 = ON_1$ to be known. From H_1 proceed horizontally to meet the Q curve, then vertically to the 45° line, then horizontally to meet the vertical through H_1 in M_1. Then M_1N_1 represents to scale the rate of spillway discharge at this position, and P_1N_1 represents the rate of inflow. The difference between these or P_1M_1 is the rate of increase of amount stored.

Take the next position N_2 so that N_1N_2 is equal to the small time interval $t_2 - t_1$ which we have decided to use, and draw the vertical line N_2P_2.

We want to find the position of H_2. The construction is as follows :—

At a horizontal distance OV from the origin O equal to $\frac{ln}{km}(t_2 - t_1)$ divisions draw a vertical line VW downwards from the time axis. From H_1 proceed horizontally to meet the A curve then vertically downwards to meet the horizontal axis in K_1, and continue this line vertically beyond K_1.

With dividers take the distance P_1M_1 and find L_1 on the vertical through K_1 so that $K_1L_1 = P_1M_1$.

The oblique line OL_1 cuts the vertical line VW in the point W_1 such that $VW_1 = h_2 - h_1$ if the scales are appropriately chosen.

Then H_2 is chosen on N_2P_2 so that $N_2H_2 = N_1H_1 + VW_1$.

Thus H_2 is approximately known.

By drawing a line horizontally through H_2 to meet the Q curve, then vertically to the 45° line and then horizontally, we find M_2.

The area $M_1P_1P_2M_2$ represents approximately the quantity stored in the time interval $t_2 - t_1$ or $\int_{t_1}^{t_2}(P - Q)dt$. From the construction this is approximately equal to $A_1(h_2 - h_1)$ which approximately represents $\int_{h_1}^{h_2} A\,dh$, since $h_2 - h_1$ is small, and A changes very little in that interval.

For greater accuracy, at the end of each step we should go through the procedure again using the mean value of H_1 and H_2 to give the intersection with the A curve, and the mean height between P_1P_2 and M_1M_2 to give the value of K_1L_1.

Thus a revised value of VW_1 is obtained leading to a more accurate position of H_2.

For trial values of the spillway dimensions, however, this refinement is hardly necessary.

It is to be understood that the graphical process is to be carried on step by step in this manner for equal small intervals of time.

After reaching a certain stage it will be found that M lies above P, indicating that the maximum water level has been reached in the reservoir, and that water is now being withdrawn from storage by the spillway discharge. The height thereafter falls off unless a further rise in the flood curve occurs.

As soon as the height exceeds the maximum permissible the computation may of course be stopped.

When it is desired to try a different width of spillway it is necessary either to modify the curve for Q; or, if Q is proportional to the width, the existing curve for Q may be used provided an appropriate oblique line through the origin be used in the construction instead of that drawn at 45°.

As a variant to the graphical method just described, a simple modification of the construction may be employed in which the steps proceed by equal increments δh instead of by equal increments of t.

Alternatively we may adopt a tabular method. First of all we require a table or curve for P against t. We also require a table or curve for Q against h, and a table or curve for A against h.

We have to solve approximately the same equation, namely,

$$\int_{h_1}^{h_2} A\,dh = \int_{t_1}^{t_2} (P - Q)\,dt.$$

We choose a convenient small interval δt or $t_2 - t_1$, and as we know the initial value of h when $t = 0$ we can proceed as follows:—

It is convenient to arrange the work in seven columns, containing t, h, P, Q, $P - Q$, A, δh.

Let us suppose that we know the figures in any horizontal row: we get the next row thus:—

Obtain the new value t_2 of t by adding δt to the former value t_1 and enter in column (1). Add δh from column (7) to h_1 of column (2) and we obtain h_2 which we enter in column (2). From the P, t table or curve choose P corresponding to t_2 and enter in column (3).

From the tables or curves we choose Q and A corresponding to the value h_2 and enter in columns (4) and (6). Column (5) is got by subtracting the entry in column (4) from that in column (3).

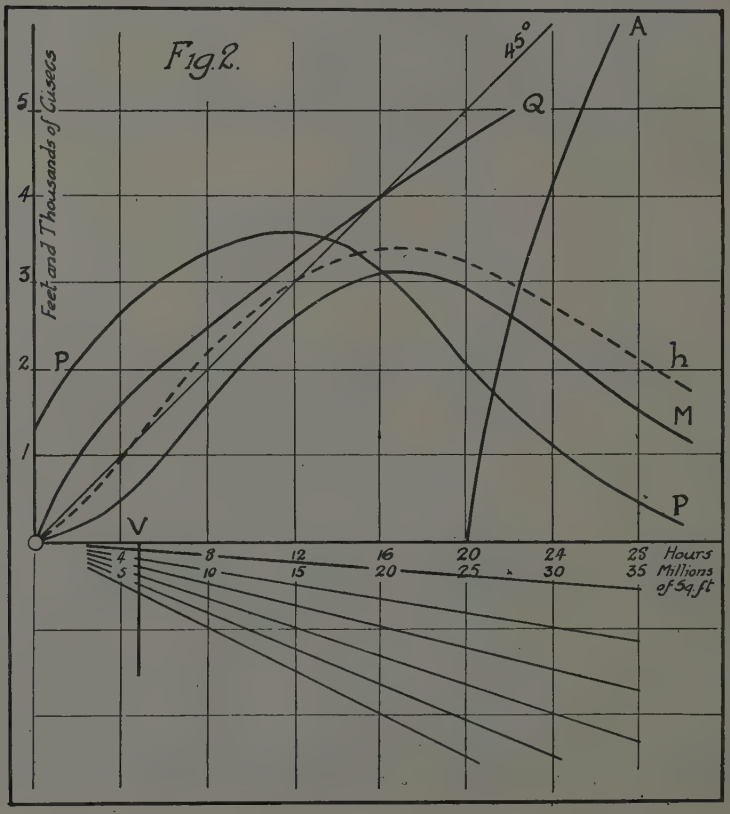
Divide the entry in column (5) by that in column (6) and multiply by δt and

we obtain δh which we enter in column (7). By adding this figure (δh) to the entry for h in column (2) we get the value $h_2 + \delta h$ or h_3 for the following row.

We proceed thus step by step from the outset when corresponding values of t and h are known. As before, the accuracy depends on the smallness of the interval δt. As a refinement we may at the end of each step revise it, using the mean values for the interval instead of the preliminary values, as explained already in connection with the graphical method.

Example: taking $\delta t = 5760$ secs.,

t	h	P	Q	$P - Q$	A	δh
0	0	1300	0	1300	25,000,000	0·300
5760	0·300	2000	100	1900	25,200,000	0·433
11520	0·733	2480	320	2160	25,500,000	0·488
17280	1·221					



It will be seen that both the graphical and tabular methods are quite rapid and straightforward, and give good accuracy. The method may be applied to various widths and depths of spillway until a satisfactory result, in which the water level rises to but does not exceed the desired height, is obtained. When this result is arrived at by larger time intervals at first, a more refined determination with smaller values of δt may be made subsequently, if desired. Obviously the case of a siphon spillway can be dealt with in a similar manner since its rate of discharge is related to the head according to a known formula.

As an example the graphical construction is given in the following case (fig. 2).

Data :—$Q = ah^{\frac{3}{2}}$; $a = 500$ foot sec. units (i.e. for a rectangular weir 150 ft. wide); $h_{\max.} = 5$ ft. ; $P_{\max.} = 3600$ cusecs; $t = 30$ hours total time of flood; A at sill level = 25 million sq. ft. = 575 acres; curves for P v. t and A v. h as shown. Convenient scales are 1 division to 0·1 ft., or 100 cusecs, or 0·5 million sq. ft., or 1440 secs.

$\therefore\ k = 0{\cdot}1,\ l = 100,\ m = 500{,}000,\ n = 1440.$

$$OV = \frac{100 \times 1440}{0{\cdot}1 \times 500{,}000}\,(t_2 - t_1) = 2{\cdot}88\,(t_2 - t_1) \text{ divisions.}$$

Take $t_2 - t_1 = 4$ divisions (say), i.e. 96 minutes.

$\therefore\ OV = 11{\cdot}52$ divisions.

Starting from $t = 0$ when the weir commences to come into operation and $h = 0$, we find graphically the curve for h v. t.

The maximum height above weir-sill is found to be 3·4 ft. for a weir width of 150 ft.

If 5 ft. submersion is allowable we should try again with a smaller width.

No. 15.

CYTOLOGICAL STUDIES OF POTATO PLANTS AFFECTED WITH CERTAIN VIRUS DISEASES.

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(Plates 4 to 8.)

[Being a Paper read before the Special Bi-Centenary Scientific Meeting of the Royal Dublin Society, June 24th, 1931.]

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Introduction.

The present work contains an account of a cytological examination of potato plants affected with various virus diseases, which was undertaken with the object of adding further to the knowledge concerning the microscopic features displayed by virus-infected plants, and more especially to investigate intracellular inclusions. Although the histological abnormalities which underlie the mottling characteristic of mosaic diseases are known in a general way, an examination of the literature shows that comparatively few workers have paid special attention to this question, and that their results show considerable disagreement. Much more attention has been paid to the occurrence of abnormal protoplasm-like cell inclusions, partly no doubt because somewhat similar bodies are associated with certain animal virus diseases, *e.g.*, the well-known Negri bodies of rabies and the Guarnieri bodies of small-pox, and partly because the hope of finding an ætiological agent is at present largely bound up with these structures. Since the pioneer work of Iwanowski (30) cytological studies of the tobacco plant in this connection have been many, but of the potato few, with the exception of the work of Hoggan (23) in America.

It was considered desirable to take up the study of the potato mosaics again for a number of reasons. In the first place, it must be borne in mind that the European equivalents of the numerous types of potato mosaic described in America still necessarily remain undetermined,[1] with the result that conclusions concerning the latter cannot be applied here without independent study. It was, therefore, considered desirable to determine if X-bodies (to use the con-

[1] Unpublished work carried out in this laboratory has shown that the American virus-diseased and healthy potato material available here contained a latent virus or viruses, the fatal effects of which on the test varieties used precluded an exact comparison.

venient term of Goldstein), or other inclusions, also occurred in the various mosaic diseases of the potato which are generally recognised as occurring in Europe, and were absent in healthy (*i.e.*, virus-free) plants. The idea also underlay the work that the bodies might possibly provide a means for the more certain differentiation of these diseases.

Material.

This study was made possible by the fact that there were available in the Department of Plant Pathology of the College stocks of healthy plants and plants containing apparently pure diseases. All the experimental material was grown in insect-proof glass-houses, and had been so grown for years. The term "healthy plant" means a plant which not only has consistently appeared healthy, but which fails to produce symptoms of virus disease when grafted on, or when its sap is inoculated into, chosen test plants. The stock of simple (or mild) mosaic used was isolated in Ireland, and has been propagated under insect-proof conditions since 1925. It has been compared with simple mosaic from Holland at Glasnevin, and at Wageningen by Quanjer, and both diseases have been found identical, and apparently unmixed. The stock of crinkle used was isolated in Ireland in 1922, and has been grown under insect-proof conditions since 1924, during all which time it has not varied. It has been compared both at Glasnevin and at Wageningen with crinkle of Dutch origin, and the two diseases have been found identical, both producing the same markedly varied series of reactions on different varieties. The stock of interveinal mosaic used is of Dutch origin (obtained from Professor Quanjer in 1926), and has been similarly propagated to the above. It has never varied or shown evidence of mixture with other virus diseases. An entirely similar disease has been isolated in Ireland. The stock of aucuba mosaic used was isolated in Ireland in 1924, and has been grown under insect-proof conditions since, without variation or evidence of impurity. It has been compared, both at Glasnevin and at Wageningen, with aucuba mosaic of Dutch origin, and both have been found identical. The stock of streak used was isolated in Ireland in 1927, three previous isolations having died out. It has not been compared with streak from other sources, but the symptoms on President agree with the symptoms of streak on this variety, as described in Holland.

The following diseases were chosen for investigation:—simple mosaic, interveinal mosaic, crinkle, aucuba mosaic, streak, and leaf-roll. Plants grown from diseased tubers were used in all cases except that of streak.

The variety President was chiefly studied, but Arran Victory, Majestic, and Irish Chieftain (for crinkle) were also used. It has not been possible to study every portion of the diseased plant in each case, and attention has, so far, been directed chiefly to the foliage and stems. Examination of tubers and roots is only in the preliminary stage.

METHODS.

Observations were made on living and on fixed and stained material. For the vital examination strips of epidermis and hand sections of leaf or stem were mounted in isotonic sugar solution or in distilled water. Neutral red was employed for vital staining when required. It colours the vacuole pale pink, thereby showing up the cytoplasm clearly (a red coloration of the nucleus or cytoplasm indicates death). It has the advantage of being comparatively non-toxic.

In the making of permanent preparations Regaud's fixing fluid, consisting of 80 parts of a 3 per cent. solution of potassium bichromate mixed with 20 parts of 40 per cent. neutral formalin, was principally employed. The material was immersed in the fixing fluid, in which it was cut rapidly into very small pieces (about 3 × 1 mm. in the case of leaf tissue) by means of a sterile safety razor blade. The fluid was allowed to act for 4 days, being renewed each day, after which the tissues were rinsed with 3 per cent. potassium bichromate, followed by post-chroming for 8–15 days in the latter solution. This was followed by washing and embedding in the usual way.

Nemec's modification is shorter, and gives good results. The solution consists of 25 c.c. of 1 per cent. chromic acid, 25 c.c. of 1 per cent. potassium bichromate, and 4 c.c. of neutral 40 per cent. formaldehyde solution, in which the material remains for two days.

Other fixing fluids, such as *Meves'* (Osmic acid and chromic acid), *Schaudinn* and *Carnoy* were occasionally used.

As a matter of routine, samples of all material were stained with Regaud's or Heidenhain's iron alum hæmatoxylin, and counterstained with erythrosin. The iron alum solution (5 per cent.) was allowed to act for 24 hours, the hæmatoxylin for 24 hours, after which the stain was differentiated with 3 per cent. iron alum, and the preparation was counterstained. By this method chloroplasts, nucleoli, and mitochondria are stained black, while cytoplasm is pink. Starch grains are not coloured.

Another useful staining method is that of Kull, using acid fuchsin (20 grms. in 100 c.c. aniline water) and toluidine blue (0·5 per cent.), and differentiating in aurantia (0·5 grams. in 100 c.c. 70 per cent. alcohol). Chloroplasts and mitochondria take a red colour, and starch becomes pale blue. A number of other stains were also used for the examination of the X-bodies. Sections were cut at 4μ and 6μ.

The procedure was to examine healthy leaves of different ages, and then to record any variation found in corresponding leaves from diseased plants.

RESULTS.

The general modifications observed are as follows. (Inclusion bodies will be dealt with in detail in a separate section):—

Simple Mosaic (President and Arran Victory).—The mottle is of a mild

nature, and internal modifications are correspondingly slight. It is difficult sometimes to distinguish the pale areas in sections of fixed material except by a slight under-development of the plastids and the occurrence of an occasional X-body, appearing as a small mass of vacuolated cytoplasm, usually close to the nucleus. In fresh material the chloroplasts of the pale areas are seen to be slightly smaller, and not quite so deep a green, as those of the green areas. Otherwise the leaf appears normal.

Interveinal Mosaic (President, Majestic, and Arran Victory).—The chlorotic areas are somewhat thinner than the green. This seems to be due to under-development, since the cells generally are not so large, and there is a smaller amount of intercellular space in the mesophyll. In a mature leaf the normal green areas were found to measure 280μ in thickness, and the yellow areas 200μ.

In young mottled leaves there is an evident retardation of development of the chloroplasts in the pale areas, and, in addition to being slightly smaller than the normal, their relative starch content is higher. In older leaves the chloroplasts of the chlorotic regions are also of a paler colour, and of more granular structure and irregular outline than those of the green areas. In general, too, they are smaller, measuring 3·7μ on the average, compared with an average of 6·5μ for the chloroplasts in the green portions of the leaf. In many cells of the light areas, however, the plastids may be almost as large as those in the green. Such variations in size, no doubt, depend on whether the plastids were affected at an early stage, or whether they had attained a considerable size before being influenced by the virus. In the former case, although the plastids continue to grow, their development is evidently retarded from the beginning, and they never reach normal size. The pathological effect in the latter case is evidenced by physical and chemical changes, resulting in the paler colour of the chlorophyll, the more granular structure of the plastid, and its reduced staining capacity.

In leaves of young diseased plants, even though the symptoms of disease are obvious, X-bodies may be difficult to find without the aid of fixed material, but under the prolonged influence of the disease they become numerous and conspicuous all through the chlorotic regions of the leaves.

Crinkle (President and Irish Chieftain).—The modifications are similar to, but more pronounced than, those caused by interveinal mosaic. There is a distinct retardation in the development of the cells in the chlorotic areas. The palisade cells are very short, and in the spongy parenchyma the cells are closely arranged, with little intercellular space. In the dark green shining areas, the leaf is thicker, and the palisade cells are longer than normal, measuring on the average 70μ × 18μ, compared to 58μ × 17μ for the palisade cell of a corresponding healthy leaf. They are also very closely set together, which, together with the reduced amount of intercellular space, may perhaps account for the glossy appearance of the leaf. The measurements given below were taken from freehand sections of a leaflet of President diseased with crinkle, the dimensions of which were 4·6 × 3·8 cms. This leaf had the usual symptoms, being

puckered, with flat pale areas interspersed with dark green bulging ones. The dimensions of a corresponding healthy leaf are given for comparison.

	Thickness of leaf.	Length of palisade cells.
Yellow area of diseased leaf	113μ	45μ
Dark green shining area of diseased leaf	137μ	57μ
	148μ	72μ
Healthy leaf	140μ	52μ
	147μ	56μ

The diseased leaves as a whole are much smaller than the corresponding leaves of a healthy plant, due to the fact that the veins of the lamina (as well as the cells of the chlorotic areas) evidently do not develop at the normal rate, and the more rapid growth of the cells in the green areas of the leaf naturally results in a puckering.

A large number of the chloroplasts in the chlorotic areas of the leaf are affected at an early stage, and appear under-developed. The chloroplasts are pale, and the amount of starch they contain is noticeably great at certain times. The accumulation of starch in the chlorotic areas of mosaic-infected leaves was observed by Woods (57), Hoggan (23), and others. X-bodies are similar in structure and distribution to those in interveinal mosaic. Corresponding to the severer disease symptoms they are more abundant, and plainly visible, in quite young leaves. In a badly-diseased leaf they are present in practically every parenchymatous cell. Tannin precipitates are fairly abundant in the yellow areas. Tannin is precipitated by the potassium bichromate in the cell vacuole in the form of large or small yellow grains, or as a fine brownish network. Phenolic substances are, of course, widespread in healthy as well as diseased plants, for heavy vacuolar precipitates are observable in the growing points of potatoes (healthy or diseased); and the epidermal cells of the leaves (more especially the upper) are generally filled with a yellow or brownish mass precipitated by the bichromate. Such precipitates (as well as others not necessarily tannin) may be found to a greater or less extent in the cells of the palisade and spongy parenchyma also, depending upon their age, but the cells of the chlorotic tissues of the diseased leaf appear to have an unduly high tannin content.

Aucuba Mosaic is characterised by a very distinct mottling of the leaves, which develop spots varying from yellow green to bright yellow in colour, similar to those of the variegated laurel.

Sections of young leaves which were not mottled were examined, both in

fresh and fixed material. The cells or cell contents did not display any abnormality. In young leaves showing early stages of mottling it was found that the chloroplasts of the light areas were somewhat under-developed; their pigment was of a paler green colour, and they showed a tendency to accumulate starch. These effects, however, were not very pronounced.

An examination of larger leaves displaying the characteristic brilliant yellow mottle showed that the spots are the result mainly of an abnormal condition of the chloroplasts. Hand sections of such leaves, embracing green and yellow areas, were mounted in the usual way. The cells of the green area appeared normal, with well-developed green plastids, containing small starch grains. In the transitional zone, the plastids were of a pale green colour, more granular in appearance, and sometimes irregular in shape. While many contained starch grains of the normal type, some were gorged with starch. In the definitely white areas the chloroplasts were almost colourless, and contained abnormal quantities of starch; in many cells they were in a degenerating condition, displaying large vacuoles, from which the starch had disappeared, or appearing in some cases as small empty spheres, with a minute mass of pale green or colourless granular material attached. Staining with iodine showed the almost complete lack of starch in such cells. The size of the degenerating plasts was reduced on the average to $3{\cdot}5\mu$, compared with an average of 6μ for the healthy ones.

Small refractive oil globules were very abundant. The production of fat appears to be a constant feature in aucuba mosaic, as in all the other mosaic diseases studied, for a corresponding healthy leaf never shows the same quantity. The fat is probably not a secretion, but a segregation, from the lipoid-protein complex of the cytoplasm. The nuclei, in the cells showing altered chloroplasts, remained comparatively unaffected, although attenuated nuclei were sometimes seen in the yellow areas.

Examination of a large number of slides of fixed material confirmed the observations made on living leaves, and showed the eventual destruction of the chloroplasts in the yellow areas. There was no evidence of hypoplasia in the chlorotic regions. The chloroplasts in the green parts seemed normal in appearance and size, and they stained deeply with hæmatoxylin and acid fuchsin. They contained a number of small, compact starch grains.

In the transitional and yellow areas the chloroplasts were seen in various stages of pathological change. Fig. 35 B, from fixed material, shows chloroplasts from one of these zones which are equal in size to those of the green area, but which show a more granular structure, and stain much more lightly with hæmatoxylin and acid fuchsin. Within the plastids are relatively large spaces. The central region of these spaces stains a diffuse blue with toluidine, and brown with iodine. The starch is not present in the form of small compact grains, as in the apparently normal chloroplasts of adjoining green areas. The conclusion has been reached that coincident with changes in the chlorophyll pigment there is an abnormal accumulation of starch, following which a process of

degeneration sets in. Eventually, however, the starch gradually disappears, leaving large spaces in the plastid, which itself begins to break up.

The condition shown in Fig. 36, in which the plastids appear to have broken asunder, may represent the next stage to that shown in Fig. 35 B. They no longer contain starch, and are merely empty spheres, with a certain amount of plastid substance attached. Further steps in degeneration are represented in Fig. 37, which shows irregularly-shaped masses of the original plastid substance. Such are the conditions which are reflected in the perfectly yellow, or almost white, spots of the badly-diseased leaf.

Mitochondria are present (and apparently unaffected) in the cytoplasm of cells showing degenerating chloroplasts. Associated with these chloroplasts a number of small spherical bodies have been seen, which stain with hæmatoxylin. They sometimes appear to be in the plastid, and sometimes in the surrounding cytoplasm (Fig. 38). They are distinguishable from the dot-like mitochondria by their larger size (average 1·5–2μ), and were regarded with interest on account of the possibility of their being foreign organisms, since Eckerson (14) has described flagellate organisms in connection with disintegrating plastids of mosaic-diseased tomato plants. No definite structure, however, could be observed in these bodies, or any resemblance to flagellate organisms.

Fig. 33 is a drawing of a living palisade cell from a leaf showing aucuba mosaic. It was completely filled with rounded, or somewhat kidney-shaped, clear hyaline masses, which gave the blue-black starch reaction with iodine. The adjacent cell on one side contained normal green plastids, and on the other side a mass of disintegrated plastids, so that the cell in question was in the vicinity of a chlorotic spot. Similar cells were found in hand sections of a leaf showing interveinal mosaic—quite isolated, and surrounded by cells containing green chloroplasts.

Again in fixed material, a slide from a leaf showing crinkle exhibited this phenomenon. The preparation was stained with acid fuchsin and toluidine blue. The chloroplasts were coloured red by the fuchsin, but one isolated cell was filled with blue-staining starch masses, and stood out conspicuously from its neighbours. In the fixed material a very fine membrane was visible, encircling each starch mass, evidently representing all that remained of the original plast. Although this condition has not been seen in healthy plants, one would not at present be justified in regarding it as a constant characteristic of diseased tissues.

When first seen in aucuba mosaic it was thought that this phenomenon might be associated with the development of the chlorotic spots. However, on finding similar isolated cells in the interveinal mosaic and crinkle material, entirely surrounded by cells containing normal chloroplasts, and not in association with the groups of cells forming the chlorotic areas, it was decided that the condition was probably not a stage in the formation of these areas. Possibly it is not a characteristic feature of mosaic-diseased leaves, but nevertheless it may present a point of interest.

Streak (President).—A cytological examination of fixed material only has been

made. The effects of streak on the foliage of this variety are very severe indeed. Small, pale areas appear on the young leaves, somewhat similar to a mosaic mottle,[2] but they very rapidly become necrotic, with fatal results to the leaf, and eventually to the plant.

In sections of young leaves showing the evanescent mottle, no striking difference marks off the pale from the green areas, but it can be seen that in certain regions the chloroplasts are rather small, and stain lightly. The cells and intercellular spaces in these areas are not so well developed, thus probably accentuating the paler colour. The mottling is soon followed by necrosis.

Examination of leaf sections shows that the necrosis generally appears in the region of the subsidiary veins, in which an enormous amount of yellow granular material, presumably of the nature of tannin, is produced. The surrounding parenchymatous cells have been obviously stimulated to abnormal development (Fig. 48). They are large, of abnormal shape, and their growth closes up the intercellular spaces. They contain small degenerate chloroplasts. Their cytoplasm is vacuolate, and tends to stain deeply. The cells are filled with a fine granular precipitate, and drops of yellow material are seen adhering to the cell walls. Apparently, the affection rapidly spreads from the vein to the surrounding mesophyll cells, which quickly become necrotic, and die. Finally, certain areas of the leaf are found to be completely dead, corresponding to the brown spots seen macroscopically.

In the petiole and stem severe necrosis also develops. The pathological changes originate usually in the internal phloem groups, which become filled with a yellow granular or plastic substance. The surrounding parenchymatous cells are seen to be large and elongated radially, so that they crush the cells between, and there is usually an attempt at cork formation around the affected areas. The necrotic affection spreads to the surrounding tissue. The brownish material appears in the intercellular spaces and between the cell-walls, which are somewhat swollen. These swollen walls give positive results when tested for suberin and cutin with sudan III, and hydrochloric acid and phloroglucin. Coincident with this is a change in the protoplasts of the affected cells. The cytoplasm, normally clear, and containing dot-like mitochondria, stains deeply, and the cells eventually shrink up and die. The necrosis may eventually embrace all the tissues, including the pith and epidermis. The xylem, owing to its position, is soon affected. When this happens, the parenchymatous tissues are found to contain yellow or brown material, and many of the cells are in a state of collapse. The walls of the vessels are dark brown, and swollen, and some of them contain shining yellow granular material adhering to the walls, whilst in others are peculiar dark brown or grey plastic masses. In certain parts of the cortex the collapse of the cells results in the formation of lysigenous cavities.

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[2] There is some evidence now available from other experiments that a mild mosaic may be present in this stock in addition to streak.

Small homogeneous vacuolate intra-cellular bodies (X-bodies) were seen in the chlorotic areas of leaves which showed the mottled condition, and before the onset of necrosis.

Leaf Roll (Arran Victory and British Queen). — A comparatively small number of sections from leaf-roll material was examined for comparison with the mosaic diseases. Slides were stained as usual with hæmatoxylin and erythrosin, and also with acid fuchsin and toluidine. Hand sections of fresh material were cut from the variety British Queen.

In a young plant not yet showing the characteristic rolling of the leaves, the chloroplasts appear normal, and contain the usual small starch grains embedded in the green plast, but as the plant develops and the disease symptoms appear in the leaves, the starch accumulates in the plast—first, in certain areas of the lamina, and then gradually all over it. When this stage is reached the chloroplasts are so full of starch that they may eventually burst. This is the condition in the brownish edges of the brittle, rolled leaves. Droplets of fat are conspicuous in the cytoplasm of living cells containing the swollen chloroplasts. Large storage starch grains are found in the parenchymatous cells of the midrib of the leaf, and in greater quantity in the ground tissue of petiole and stem.

The palisade cells of the leaf are enlarged beyond the normal size, and the intercellular spaces in the spongy parenchyma are greater. Vacuolar precipitates are present in the spongy mesophyll and palisade cells in greater abundance than in a corresponding healthy leaf.

X-bodies were not observed in any of the sections. Tsen-Cheng (55), who recently made an extensive cytological study of this disease, did not report any unusual cell inclusions.

INTRA-CELLULAR INCLUSIONS.[3]

In this portion of the work particular attention was paid to the so-called X-bodies. They were studied in living, as well as in fixed and stained material, and were found to be present in simple mosaic, interveinal mosaic, crinkle, and streak plants which displayed a mottle, but they were not found in aucuba mosaic or leaf-roll. X-bodies did not occur in healthy plants.

The inclusions appear to be of similar nature in the four mosaic diseases mentioned. They are most numerous, however, larger, and more conspicuous generally, in leaves affected with interveinal mosaic and crinkle which have reached a certain stage of development. One can examine leaves from young plants attacked by either of these diseases and have difficulty in finding X-bodies, either in living or fixed material, but as the plants become older the bodies are found in increasing numbers and in varying stages of development.

[3] It is recognised that this term, although in general use, may not be altogether suitable, as it conveys the impression that the structures described under this heading are necessarily foreign. (See Rivers (51), p. 113.)

X-bodies in living material.—In suitable material the vacuolate bodies may be conveniently examined in the living state, by stripping pieces of epidermis from the lower surface of the leaf lamina or midrib, and mounting them in isotonic sugar solution or water. It is possible also, but less convenient, to obtain good preparations from hand sections of the leaves.

In a normal epidermal cell the cytoplasm is confined to a thin layer lining the cell wall and to a number of fine cytoplasmic strands traversing the large central vacuole. The nucleus may be situated in the parietal layer or suspended by strands in the centre of the cell. A number of chloroplasts are present in the cytoplasm. Numerous crystals and small hyaline particles displaying Brownian movement are present in the vacuole. The crystals are mainly calcium oxalate, and are dissolved by dilute hydrochloric acid. In the normal hair cell also there is a large central vacuole containing crystals and grains in varying quantities, and a few small chloroplasts lying in the cytoplasm.

In the diseased cells, if the material is suitable, the X-bodies form conspicuous vacuolate masses, usually closely associated with the nucleus. As already mentioned, however, a young diseased plant, although showing clear symptoms of mosaic, does not generally display these conspicuous vacuolate bodies.

Many living epidermal and hair cells from such comparatively young plants have been examined. As in healthy cells, abundance of crystals and small particles displaying Brownian movement are present in the vacuole. The first visible abnormality is the presence in the cytoplasmic strands of a rounded protoplasmic mass (Fig. 6); while one is the usual number present, 4 or 5 of these accumulations, varying in size from 3·5μ to 9μ in diameter, have been seen in a cell. They are carried round in the rapidly-flowing strands, sometimes quickly, sometimes slowly, and often remain stationary for a time in the primordial utricle. They also seem to rotate slowly, and change shape somewhat, becoming more drawn out, but these movements are not necessarily autonomous, and could be caused by irregularities in the rate of flow of the streaming cytoplasm. They look like rounded masses of host protoplasm, which acquire a less fluid consistency than the general cytoplasm of the cell. They appear somewhat more coarsely granular than the cytoplasm of the strands. The masses have no apparent connection with the nucleus, and while they may impinge upon it they are usually carried on again by the protoplasmic current. As the motion of the latter slows down, however, the mass often comes to rest beside the nucleus, as though the current were insufficient to draw it past. While in a few cases vacuoles were apparent they were not clearly visible in living material, as a rule, in these small bodies.

In plants suffering from the more prolonged effects of disease the cells display much larger and more conspicuous masses, situated in the cytoplasm, and usually in juxtaposition to the nucleus (Figs. 8–10). The presence of a variable number of vacuoles is a characteristic of these bodies. Quite commonly there is one large vacuole and a number of small ones. Within them small

granules displaying Brownian movement are sometimes seen (Fig. 1). When well developed the bodies reach a diameter of 30μ or more, and they may be much larger than the nucleus. Sometimes they envelop it almost completely, but they are never incorporated with it; and, while the nucleus may be slightly indented, due to the juxtaposition of the mass, its membrane always remains intact. These conspicuous vacuolated bodies are not carried round the cell, but shift position only to the same extent as the nucleus.

They usually have a definite outline, suggesting the presence of a membrane. The shape varies a great deal. The rounded form predominates, but in cells which are much longer than they are broad, such as those of the parenchyma of the midrib (Fig. 7), the bodies are generally elongated.

As the development of the bodies proceeds they are characterised by an accumulation of conspicuous globules. These appear to be loosely attached to the surface of the body, as well as being present in its substance. Detached globules of a similar nature may be seen scattered in the surrounding cytoplasm. Their presence often masks the vacuolated structure of the body, and gives it a very coarsely granular appearance.

A number of microchemical tests were performed on the X-bodies. Indophenol blue[4] was used intravitally as a test reagent for fat. The compound is obtained in the nascent state, and as the medium is water there is an obvious advantage over other fat stains, which require to be dissolved in alcohol or other organic solvents. It dissolves readily in fat, colouring it a deep blue.

When applied to cells containing X-bodies it had no effect on the general matrix of the body, but the conspicuous globules were stained a clear blue, indicating their fatty nature. Any other fat droplets present in the cell were also stained, *e.g.*, in epidermal cells, each of which usually contains one very large oil globule.

With Millon's reagent the X-bodies, nuclei and cytoplasm were coloured yellow. With caustic potash and dilute copper sulphate (Biuret test), the X-bodies and nuclei showed a violet-pink colour. With saturated aqueous solution of picric acid, the X-bodies and nuclei also became a deep yellow colour. The X-bodies were found to be insoluble in alcohol, acetic acid, and nitric acid. They shrank but did not dissolve in 75 per cent. hydrochloric acid.

When preparations were kept in a moist chamber for 2 days the bodies did not disappear, but became granular, like coagulated cytoplasm.

From the appearance of the bodies in the living state, their reaction to chemical tests, as well as their staining properties in fixed material, it is evident

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[4] The solution must be freshly made up and is prepared as follows:—

(*a*) To 100 c.c. water, warmed over a water bath, add 0·5 grms. thymol, and at the moment of fusion add 0·5 c.c. of a 33 per cent. solution of potassium hydroxide. This solution keeps for several weeks.

(*b*) Dissolve 0·5 grms. of dimethyl-paraphenylene-diamine in 50 c.c. water, and carefully decolorise by addition of potassium hydroxide drop by drop.

For use mix equal quantities of (*a*) and (*b*) and dilute with 10 times the volume of distilled water.

that the matrix of the X-body is protoplasmic. The conspicuous refractive globules are fatty bodies, adhering to, and also embedded in, the cytoplasmic mass. The latter are not seen in material fixed by Regaud's method, as they are dissolved away during the process, leaving the homogeneous vacuolated substratum clearly exposed. These globules are interpreted as fat droplets arising from the breakdown of the lipoid-protein complex of the cytoplasm as a result of the pathological condition of the cell. As already mentioned, even in the early stages there appeared to be an abnormal amount of fat in the cytoplasm of the diseased cell.

As the bodies become older they have often a yellow tinge, and in some cases the vacuoles of the X-body contain a brown substance. In the epidermal cells of leaves from severely diseased crinkle and interveinal mosaic plants, elongated crystalloid structures have been observed, often stretching right across the cell, which appear to be situated in the cytoplasm. They stain yellow with iodine. They do not appear until the disease is advanced, and are regarded as evidence of protoplasmic degeneration.

An interesting type of body was seen on two occasions in hair cells of Irish Chieftain leaves affected with crinkle, and is illustrated in Fig. 12. This body, about 11μ in diameter, consisted of a rounded mass of protoplasm, containing numerous small granules and fat globules, and surrounded by a fine membrane. It was free in the cell vacuole, having no apparent connection with the nucleus or cytoplasmic strands. During the six hours it was under observation the protoplasmic contents were in a state of continual agitation, and the body displayed a constant pulsating and twisting motion. It changed its position slowly, possibly as a result of the twisting movement. At times a clear area appeared at one side of the body (Fig. 12 B), due either to a contraction of the protoplasmic contents or to a protrusion of the surrounding membrane. This type of body probably cannot be associated with the X-bodies already described on account of its rare occurrence, and because it appeared to be quite definitely an organism of some kind rather than a product of the host-cell cytoplasm. Unless its presence should prove to be more widespread, however, the possibility of its being a pathological agent in virus diseases is remote.

One other abnormal structure has been seen in the living cells in crinkle and interveinal mosaic. In occasional hair cells colourless striated masses occur, which appear to be formations in the cytoplasm. They are not common, and have not been found in fixed material (Fig. 11). Similar bodies, however, have been observed on a few occasions in hair cells of tobacco infected with potato mosaic. They resemble the striated bodies described by Goldstein (15, 16) and others in connection with tobacco mosaic. and they are similarly resolved into a shapeless mass by the addition of dilute hydrochloric acid. In ordinary tobacco mosaic these striated bodies are, according to all reports, very abundant. Goldstein states they are faintly but clearly distinguishable in living material, but are clearly marked in fixed preparations. It is considered that the striated bodies seen here are similar to those occurring in tobacco mosaic.

X-bodies in fixed material. — In preparations from leaves showing simple mosaic there was found only an occasional small vacuolate body, resembling cytoplasm in structure and staining properties (Fig. 13). A number of sections showed no abnormal inclusions. Similarly, in leaves affected with streak which displayed the mild mottle preceding necrosis, the vacuolated bodies were few and small. Material taken at intervals from plants affected with interveinal mosaic and crinkle showed a progressive increase in number and size of the bodies with the more prolonged effect of the diseases on the plant.

Since no material differences were observable in the inclusion bodies associated with these four mosaic diseases—apart from the question of number and size—it will not be necessary to give a separate account of them in each disease. Most of the following observations refer to interveinal mosaic and crinkle, which provided the best material for study.

With iron alum hæmatoxylin, counter-stained with erythosin, the X-bodies stain pink, like the nucleus, or just a shade lighter. The nucleus is always clearly distinguishable, because, as already mentioned, it never becomes incorporated with the body. The nucleoli and mitochondria are black, and the chloroplasts black or dark grey. This was the most widely used staining method, as it was found the most reliable. With acid fuchsin the bodies are unstained, but the mitochondria within them stain red.

In fixed material the first abnormality which can be observed in leaf sections is the presence in the cells of small homogeneous masses of protoplasm staining pink with erythrosin, and characterised by being vacuolated. They are usually round or oval in shape, and lie close to the nucleus, but they may form quite irregular masses, stretching across the cell (Figs. 14, 15). They do not seem to have any organised structure, and give the impression of being cell cytoplasm which has accumulated in a certain region of the cell. They have not the appearance of foreign elements. In more advanced material, however, they are seen to be rounded off (cf. Hoggan), and appear as well-defined "bodies," which, although lying in the cell cytoplasm, do not appear as actually part of it (Figs. 16, 17, 40, 41).

When definitely formed, the X-bodies appear as conspicuous masses in epidermal and hair cells of the leaf, in the palisade and spongy parenchyma, and in the parenchymatous tissue of the veins. They have a homogeneous or finely granular structure, and stain like cytoplasm. The typical shape is more or less rounded, but elongated and irregular shapes also occur (Figs. 25, 26). There is rarely more than one in a cell. They are situated close to the nucleus, from which, however, they are clearly distinguishable. The presence of the body seems to have little effect, if any, on the nucleus, and although the latter may be partially surrounded or indented by the inclusion, its membrane remains intact. Numerous vacuoles are present in the X-bodies—some large and some very small. The bodies vary in diameter from 6μ up to about 50μ, the average size being about one and a half times to twice the size of the nucleus.

Mitochondria are present in the X-bodies, which, as Holmes (24) points out,

is evidence in favour of their living cytoplasmic nature. Within the vacuoles of the well-developed bodies are frequently to be seen vague structures, often elongated or rod-shaped, sometimes round (Figs. 17, 22, 27). They do not stain clearly with hæmatoxylin or erythrosin, and seem to be of a light brown colour. They are apparently similar to the structures occasionally found in the bodies associated with wheat mosaic by M'Kinney (43). In some of the writer's material they are very common. Their presence possibly depends on the stage of development of the bodies. Goldstein (16) and others have also referred to structures in the vacuoles of the X-bodies, some of which stained quite definitely with the saffranin of the triple stain.

Several different staining methods were employed in order to investigate further the nature of these vacuolar contents, and also with a view to determining whether differential staining would reveal the existence of chromatophile corpuscles, other than mitochondria, within the substance of the X-body. This is of importance, because the cell inclusions in certain animal virus diseases are regarded as complex structures of dual nature, consisting of microörganisms embedded in material produced by the cell in response to their action, as illustrated by the so-called Chlamydozoa and Strongyloplasmen of v. Prowazek and Lipschütz. The following are some of the stains which were tried:—

Loeffler's methylene blue and erythrosin.—Sections were left overnight in the methylene blue, and decolorised in 0·5 per cent. acetic acid, followed by washing in water; dipped for about 5 or 10 seconds in the counterstain; rinsed in water, dehydrated rapidly in alcohol, cleared in xylol, and mounted. Nucleoli and chloroplasts stain blue; nuclei and X-bodies pink or lilac. If the methylene blue is not entirely washed out of the X-bodies the vacuolar structures are blue, but they do not retain the blue after it has been washed out of the X-body generally.

Giemsa.—One drop of the Gurr stain was added to every 1 c.c. of distilled water made alkaline by the previous addition of 1 drop of a 1 per cent. solution of potassium carbonate to 10 c.c. of water. Slides were left in the stain overnight, dipped in 50 per cent. methyl alcohol for a couple of minutes, washed in tap water, dehydrated, and mounted.

The X-bodies generally assume an even pinkish-blue colour, and the nuclei a deeper purplish-blue shade. The structures in the vacuoles tend to stain, but do not stand out clearly, and in cases in which the X-bodies take only a light pinkish stain the vacuolar contents are not coloured. There was no evidence of nuclear substance in the body.

Leishmann.—Sections were treated with diluted Leishmann (1 part stain, 2 parts distilled water for 1 hour); decolourised with acetic acid solution (1 in 1,500 aqueous), washed in distilled water, dehydrated rapidly, and mounted. The X-bodies assume a lilac tinge with this stain, and the vacuolar structures stain blue. Mitochondria remain unstained.

Basic fuchsin and methylene blue.—The stain (0·5 c.c. saturated alcoholic solution of fuchsin, 10 c.c. saturated alcoholic solution of methylene blue diluted

in 30 c.c. distilled water) was poured over the sections, and the slide was warmed till vaporisation occurred, then washed in tap water, dehydrated rapidly, and mounted. The X-bodies stain a light magenta. It was not possible to stain the structures in the vacuoles blue without overstaining the X-bodies.

Acid fuchsin and Unna's polychrome methylene blue. — Following Milovidov's method with this combination, the chloroplasts and nucleoli are stained red, and the X-bodies a blue shade with red mitochondria. The structures in the vacuoles are blue, slightly deeper than the X-body itself.

From these and other staining reactions it was concluded that the X-bodies represent a mass of cytoplasm containing the usual quantity of mitochondria. The bodies are acidophilic, except in the older stages, when there is a tendency for the reaction to be basophilic. There are no structures within the bodies which could be interpreted as nuclei. The vacuolar contents stain with methylene blue, if at all, but they are not comparable to the "inner bodies" of the Negri bodies, which seem to be clearly defined, and are said to be markedly basophilic (51). It is thought that they may be precipitates of a tannin-like nature within the body, which are described further on.

Some of the material from interveinal mosaic (variety Majestic) and crinkle (varieties President and Irish Chieftain), besides displaying numerous well-developed X-bodies of the usual type, showed further developments, including the apparent breaking-down of the bodies. In addition to the ordinary fine cytoplasmic strands which may usually be seen radiating from the nucleus and X-body, an elongated thickened structure was sometimes observed stretching across the cell, often from the X-body to the cell wall, and seeming like an appendage of the former, or more rarely apparently detached from it (Figs. 24, 25, 42). The appendage frequently has a stiff appearance, as though it were of crystalline structure, and it is characterised by being extremely chromatophile. With hæmatoxylin and erythrosin, it commonly stains deep pink along the margins, while its internal substance is quite black. Sometimes the entire structure is black, or, again, it may stain a deep pink only. The appendages stain deep blue with Loeffler, Giemsa and Leishmann, and red with acid fuchsin.

In addition to these crystalloid structures, much smaller filiform bodies (staining black with hæmatoxylin and red with acid fuchsin) have occasionally been seen lying in the cytoplasm beside the X-body, or, if the body is drawn out into an irregular shape, lying apparently within it (see Figs. 23, 26, 44). Whether they are of a similar nature to the larger and stiffer chromatophile appendages just described is difficult to say. Their counterpart has not been seen in living material. The elongated appendages probably correspond to the crystalloid bodies which were seen in the epidermal cells of living material, and regarded as crystalloid formations of albuminous nature. Presumably, they are degeneration products of the cell cytoplasm, and indicative of the moribund condition of the cell. Henderson Smith (25) saw what he described as "crystalline formations of, or in, the cytoplasmic strands." They are possibly of similar nature.

In the vital examination it was observed that the X-bodies frequently had a yellow tinge in the advanced stages, indicating a change in composition. Figs. 28, 29, and 43 represent a common appearance seen in some of the fixed material. In close association with the body may be seen a mass of brown material, resembling a tannin precipitate, giving the impression that the centre of the body had been partially liquefied and extruded, and that the resulting substance, being rich in phenolic compounds, was precipitated as a brown mass by the potassium bichromate. The bodies, while still appearing rounded in certain focal planes, have not really a spherical shape, and Fig. 29c shows an appearance which is quite common at this stage, and which looks as though division of a body had taken place. Examination, however, always showed that such was not the case, and that the bodies are merely of irregular shape, and do not really show fission figures.[5] In very advanced stages the bodies may have most irregular outlines.

The yellow or brownish shade frequently acquired by the bodies in the later stages has already been referred to, and the nuclei of cells containing such bodies take on a brownish colour also. The staining properties of the yellowish or brown bodies is affected, so that they stain a pinkish brown with erythrosin, if they take the stain at all. In other cases certain bodies, while retaining their original outline, become granular and less dense in appearance, and these, too, may take the erythrosin stain very lightly or not at all (Fig. 45). Such bodies stain more readily with methylene blue.

The conclusion has been reached that the X-bodies, originally composed of living cytoplasm, die away, or at any rate undergo serious changes, their degeneration being accompanied by the production of tannin-like substances. In cells containing degenerating X-bodies, the nuclei are also altered, as their staining reactions show, but the cells are still living, although presumably in a weakened condition. It is not the rule, indeed, for these cells to die as long as the plant survives, although certain of them may do so. It may be pointed out that Reinking (50) and Kunkel (33) both observed that the inclusion bodies associated with mosaic of sugar-cane changed in structure with increase of age, becoming more granular, and showing other alterations. Changes in the composition of animal-cell inclusions have also been noticed, *e.g.*, the inclusions of fowl-pox during their evolution become lipoidal and blacken (51).

One other feature was observed in this material. It frequently occurred that a brownish or greyish mass was situated close to an X-body, and within this mass were a number of deeply-staining spheres, each surrounded by a clear space, like a halo (Fig. 32). The spheres varied in size from 1μ to 3μ. Unless these are to be interpreted as vacuolar precipitates, they are abnormal. Usually, if the sap is rich in protein substances, black-staining precipitates will be general throughout the cell vacuole, but these chromatophile spheres seem to be localised in the vicinity of the X-body. Possibly, they may represent a precipitate from the contents of the body, but it may be that they have some other significance.

[5] The writer has no experience of the bodies in meristematic cells, but Goldstein has described division figures of X-bodies in the dividing cells of tobacco and dahlia.

Stems.—In spite of the frequency of the X-bodies in the leaf cells at certain stages, none of the stem sections have revealed similar inclusions. Furthermore, no green or yellow-green bodies resembling organisms, as described by von Brehmer (5), have been observed.

Growing Points.—Sections through terminal and axillary buds were made in the case of crinkle-infected and healthy plants, and stained in the usual way.

Typical X-bodies were not found in the meristematic cells of the diseased plant, and no distinction between it and the healthy plant was observed.

In the diseased plant, however, the epidermal and sub-epidermal cells of the leaf primordium and young stem were found to contain narrow, elongated, apparently homogeneous, structures, often stretching across the cell, and staining deeply with erythrosin. Hæmatoxylin frequently stained certain regions within them black. They appear to be similar to the structures described by Klebahn (31) as characteristic of intercostal and simple mosaic, and it is considered that they are probably masses of protein material, which separate out in this form as a result of the pathological condition of the cells.

DISCUSSION.

It has been pointed out that a review of the literature bearing on the subject indicates much disagreement amongst workers as to the nature and the extent of the modifications which underlie the mottle effects in mosaic-infected leaves. Woods (56), Iwanowski (30), Dickson (8), Cook (6), Smith (52), Goldstein (16), and many others have pointed out the lack of proper differentiation and development of the cells in the chlorotic areas of mosaic-infected leaves. Most workers, in addition, have observed pathological changes in the chloroplasts and nuclei. They refer to the smaller size and paler colour of the plastids in the chlorotic areas, and actual disintegration of the plastids has frequently been recorded—*e.g.*, by Dickson, Goldstein, and Dufrenoy in tobacco, and by Hoggan (23) in tobacco, potato, and other virus hosts. Eckerson described the progressive liquefaction and final disorganisation of the chloroplasts in mosaic-infected tomato leaves, due to the presence of mottle flagellate organisms. Sorokin (54) also described the liquefaction of the chloroplasts in tomato leaves, accompanied by the appearance of rapidly-moving hyaline bodies, which she thought resembled micro-organisms of the Rickettsia type.

Goldstein concluded that the degree of cell modification depended upon the age of the cell at the time when infection took, and that the histogenic development of the cell was interfered with as a result of contact with the virus. In the most recent paper on the subject Cook (6), who studied mosaic-infected tobacco, tomato, sugar-cane, and cowpea, emphasised the opinion that the effect of the disease agent on the cells is purely inhibitory; he found that the cells of the chlorotic areas displayed a hypoplastic condition, and their chloroplasts were smaller and fewer in number than in the cells of the green areas. With age and exposure to sunlight, however, the chloroplasts increased in size and

number, so that the mottle faded from the leaves. There was no evidence of disintegration of the plastids, nor, apparently, any pathological effect on them, beyond their slower development as compared to the plastids of a healthy cell. The conclusion appears to follow that chlorosis, being thus due to retarded development, could not appear in cells which had already developed normally, and Cook considered that the pale areas of the leaf increased in extent by division and growth of these early-infected cells, rather than by invasion of the active agent into the green areas after the tissues were mature.

The present writer's observations on mosaic-infected potato leaves also show that inhibited development is a feature of chlorosis in certain diseases, but the pathological effect, in potato at any rate, is considered to be more complex than Cook has suggested. The physical and chemical changes which may occur in the chloroplasts, the marked abnormalities in starch content in the chlorotic cells, and the fatty degeneration which is also evident in them, can scarcely be attributed to retarded development only. Undoubtedly, under certain external conditions, especially in the milder types of mosaic, the mottle may fade; in such cases the pathological effect is overcome to a greater or less extent, and presumably the metabolism of the cells, previously upset, is more or less righted. When the chlorosis is severe, as in certain varieties suffering from crinkle, the leaves never grow out of the mottle. In the case of aucuba mosaic the yellow spots are mainly due to loss of green pigment, to excessive quantities of starch in the plastids, and to alterations in the structure of the chloroplasts, which frequently disintegrate; hypoplasia of the cells is not an accessory factor, and the mottle cannot be attributed to inhibited development only. In general, it is considered that there is evidence of definite physiological disturbances in the chlorotic cells, which may set in at a comparatively late stage of the development of the latter, acting in conjunction, in many cases, with an initial reduction in the size of the cells and a general underdevelopment of their contents.

Woods (56, 57) was of opinion that the mosaic disease of tobacco was due to excess of oxidizing enzymes, which he observed to be abundant in the chlorotic areas of diseased leaves; and to this excess he attributed the destruction of the chlorophyll pigment, as well as the difficulty in starch translocation which was characteristic of the pale areas. He showed that tobacco oxidase in strong solution inhibited the action of diastase, so that starch was only changed to erythrodextrin. Allard (1) claimed that peroxidase could not be the pathological agent, since he was able to destroy the peroxidase in the sap while still retaining infectivity in the latter.

In this connection one may refer to the recent work of Lubimenko (38), on chlorophyll and its transformation in the tissues, in which he emphasises the importance of oxidizing enzymes. The quantitative and qualitative evolution of the pigments of the developing plastids bears a direct relation to the quantity of peroxidase. A medium quantity of the latter seems to be the optimum for the best development of chlorophyll. He refers, too, to the presence of a reducing substance (anti-oxidase), which regulates the oxidation of the pigments,

and accordingly protects the chlorophyll during photosynthesis against the action of light and peroxidase. It is necessary to have a delicate balance between these two reciprocal enzymes for the conservation of the green pigments. Otherwise the chlorophyll will diminish, and finally the protein body of the plastid will be destroyed.

There is evidence that disturbances are present in the enzyme mechanism of the cells of mosaic-infected leaves, and the modifications observed microscopically could, apparently, be entirely due to enzymic disturbances. Such a pathological condition would not *necessarily* be dependent upon the presence of a parasitic organism in the cells, and examination has failed to reveal the presence of any definite organism. Nevertheless, the infectious nature and other important characteristics of virus diseases are not readily explainable without postulating the agency of an organism, and, until stronger evidence to the contrary is forthcoming, the theory that the pathological agent is an ultramicroscopic organism cannot be abandoned.

The vacuolate inclusions described in this paper are evidently of similar nature to those found in wheat, Hippeastrum, tobacco, and other hosts when attacked by virus diseases. Their appearance in fixed material as finely granular or homogeneous vacuolate masses, staining like cytoplasm, and containing mitochondria, accords well with the descriptions of X-bodies in other plants given by Palm (47), Kunkel (33), Holmes (24), M'Kinney (43), and Goldstein (15), most of whom also describe the X-bodies in living material as resembling protoplasm. The only reference to potato X-bodies in living material has been made by Hoggan (23), who said that her observations on fixed material were confirmed on living material. The writer's observation that numerous fatty globules are associated with the cytoplasmic matrix of the X-body has not previously been made. Dufrenoy (10), however, obtained positive fat reactions with the vacuolate masses in tobacco. Henderson Smith (25) demonstrated by chemical tests that the bodies in *Solanum nodiflorum* are of proteid nature, and give negative fat reactions. The majority of workers have concluded from their appearance and staining properties that the bodies are protoplasmic. Sorokin (54), Kunkel (33), and M'Kinney, Eckerson, and Webb (43) appear to incline strongly to the view that the X-bodies seen by them may possibly represent a stage in the life-history of an organism, which causes the disease. They point out the resemblance of the X-bodies to Negri bodies. Lyon (40) and Reinking (50) are quite definite concerning the foreign origin and parasitic nature of the bodies in Fiji disease of sugar-cane. Goldstein, too, seems to have little doubt as to the foreign nature of the X-bodies in tobacco and dahlia, as she considered the movement they displayed to be autonomous, and her examination of growing points showed that the bodies became distributed to the daughter cells when a host cell containing a body divided. She was of opinion that they may be plastic enough to pass anti-bacterial filters, and considered that by their appearance in the cells they cannot be regarded as simple products of the host cell or cell nucleus. Likhité (37) asserted that there could be no

doubt as to the living nature of the intra-cellular vacuolate bodies in mosaic-infected tobacco, as he observed autonomous movement, division by constriction, conjugation, and sporulation. On the other hand, Iwanowski (30), Palm (47), K. M. Smith (53), F. F. Smith (52), Plakidas (48), Hoggan (23), and Henderson Smith (26) believe the X-bodies to be a product of the host cell cytoplasm, due to the irritation of the virus. Henderson Smith, however, points out that they do not occur in the cells of plants suffering from any pathological condition other than virus diseases, or in healthy plants, so that it is generally agreed that they are specific to virus diseases. Dufrenoy is an exception in so far as he states that the appearance shown by virus-infected cells may also be observed in connection with fungal and bacterial diseases. One cannot be certain, however, whether the localized chromatophile vacuolated areas described by Dufrenoy as occurring in the cytoplasm of the diseased cells (and believed by him to correspond to the X-bodies found by others in tobacco mosaic) are definitely equivalent to the X-bodies described by Goldstein in connection with this disease.

The X-bodies examined by the writer in the virus-diseased leaves of potato, in spite of their suggestive appearance, are not regarded as foreign organisms, but as a modified form of the cell cytoplasm. The absence of a nucleus, or nuclei, is one reason for rejecting the theory that they are independent organisms; on the other hand, mitochondria are present, as in the normal cell cytoplasm. The fact that the masses are never free in the vacuoles, but always connected with the cell cytoplasm, points rather to their origin from the latter than to an independent origin and existence.[6] The bodies show no evidence of a life-cycle, and no apparent reproductive phrase. They appear to end by degenerating, and to have no significance in the perpetuation of the disease. Henderson Smith observed that the bodies in *Solanum nodiflorum* crystallized out in old leaves.

The bodies bear a general resemblance to certain animal virus inclusions—*e.g.*, the Negri and Guarnieri bodies. The Negri bodies, however, have been described as acidophilic masses, containing numbers of strongly basophilic granules, but the latter are not demonstrable in potato X-bodies. The potato bodies differ from the Guarnieri bodies, too, in the fact that they are not dissolved by 1·5 per cent. acetic acid. Finally, the X-bodies appear to arise as the result of viscosity changes in the cell cytoplasm; masses of physically-altered protoplasm occur in the strands or parietal layer, and such altered protoplasm accumulates in the course of time, usually at the junction of the cytoplasmic strands with the nucleus, to form the typical X-body. The rounded form most commonly displayed by the latter is the natural shape which one would expect a practically stationary mass of cytoplasm to assume in the cell; the development of vacuoles in such a system is not

[6] The protoplasmic body surrounded by a membrane which was seen in the hair cells of Irish Chieftain affected with crinkle, resembles the living X-bodies described by Goldstein in so far as it appeared to be free in the vacuole. However, the writer cannot at present connect this body with the common and typical X-bodies.

understood, but may also be a normal physical development under the circumstances. The segregation of fat globules, which is probably an aggravation of a normal process (22), the formation of crystalloid structures of protein nature, together with the excessive production of tannin substances in the diseased cells, are all regarded as results of protoplasmic degeneration in these cells which appear to be in a state of low vitality for a long period previous to the death of the plant. It is not possible to say how these disturbances are brought about in the first instance. Alterations in hydrogen-ion concentration or osmotic pressure, for example, due to disturbed metabolism, might be responsible for physical alterations in such a colloidal system as the protoplasm, but the repeated statements that the X-bodies do not occur in connection with any pathological condition except that caused by virus diseases indicates that the disturbing factor leading to their formation is of a special and unusual character. Unless they can be produced apart from virus diseases, this can hardly be disputed. Henderson Smith has watched the building up of the X-bodies characteristic of aucuba mosaic in *Solanum nodiflorum* by the coalescence of numerous tiny particles which appear in the streaming cytoplasm a few days after inoculation, and he has suggested that these minute granules represent foci where the cytoplasm has condensed, due to the presence of an ultra-microscopic particulate virus. Hoggan came to the conclusion that the X-bodies of typical appearance occurring in diseased potatoes were formed by a "rounding-up" of irregular vacuolated masses of protoplasm-like material which were conspicuous in the chlorotic cells of diseased leaves; she did not make any suggestion as to how these irregular masses, which were sometimes very large, were produced in the cell, but she considered them to represent some modification or abnormal form of the host cell cytoplasm.

The results of the present study are in agreement with those of Hoggan as regards the origin of the potato X-bodies from the cell cytoplasm. Hoggan believes that the presence or absence of X-bodies may be useful in the identification of certain different virus diseases in cases where a single host species is susceptible to more than one virus. This conclusion was presumably due to her observation that two types of cell inclusion, namely, X-bodies and striate material, were found to be a constant feature of ordinary tobacco mosaic when inoculated into other solanaceous hosts, provided these developed symptoms of mottling or chlorosis, while certain other virus diseases produced no X-bodies in any of the hosts examined, even though a definite mottling appeared. Furthermore, when ordinary tobacco mosaic was transferred to potato the cells of the potato leaf showed abundance of X-bodies and striate material similar to those occurring in every other host plant affected with tobacco mosaic, in addition to, and readily distinguishable from, the masses of vacuolate material characteristic of potato mosaics alone. This result is interesting, as it appears to be an indication that the X-bodies produced by tobacco mosaic in potato are not formed by a "rounding-up" of the abnormal vacuolate material in the cells in the same way as the potato X-bodies.

The present writer has no proof that a particular virus disease is associated with its own peculiar type of X-body, even in the case of one particular host, such as the potato. She is not in a position to state whether the presence of a mosaic disease from another plant—for example, tobacco mosaic—would produce in the potato inclusions recognisably different from those associated with potato mosaics.

The fact, however, that certain diseases of a particular host plant result in the formation of X-bodies in the cells of that plant while others do not is of some use for purposes of classification, as it indicates that members of the first group are of a similar nature to one another, but differ in perhaps an essential way from those of the second group. From observations on the general cell modifications occurring in the potato diseases under investigation in this work, the three diseases, simple mosaic, interveinal mosaic, and crinkle, fall into one group, while aucuba mosaic seems to belong to a different category. In infection tests on other hosts, such as tobacco and Datura, aucuba mosaic also differs strikingly from the other three mosaics. Streak, when accompanied by mottle in the early stages, resembles simple mosaic, but becomes clearly differentiated with the onset of necrosis. Leaf-roll presents a consistent characteristic picture, which definitely distinguishes it from the other diseases. The microscopic features, therefore, so far as classification purposes are concerned, serve as additional evidence to support a grouping based on macroscopic symptoms, physiology, transmissibility, and other general characteristics.

Summary.

This paper is a record of microscopic studies of potato plants affected with certain virus diseases, namely, simple mosaic, interveinal mosaic, crinkle, aucuba mosaic, streak, and leaf-roll.

The modifications underlying the mottle effects in mosaic-infected leaves are described. The extent of these modifications is proportional to the disease symptoms.

In simple mosaic, interveinal mosaic, and crinkle, the chlorotic areas of the leaves are thinner than the green areas, due to the inhibited development of the cells in the pale areas.

The chloroplasts in the cells of the chlorotic areas also display inhibited development, as they are frequently smaller and fewer in number than in corresponding healthy cells. They may, however, be almost normal in size, but differ in other respects from the plastids of a healthy leaf. The tendency to abnormal starch accumulation is a feature of the chlorotic cells.

In aucuba mosaic the chlorosis is due to loss of green pigment, coupled with excessive quantities of starch in the plastids and alterations in the structure of the chloroplasts, which sometimes disintegrate.

Excessive quantities of tannin and fat are present in the chlorotic cells of mosaic-infected leaves.

The cells of the green areas of mottled leaves are similar to those of healthy leaves, except in the case of crinkle, in which case the cells of the green portions are often unusually long and narrow and closely set together. This condition is attributed to the retarded growth of the veins and of the chlorotic cells surrounding the green areas, as a result of which the green cells cannot assume the normal shape.

Streak is characterised by the development of necrotic areas in the leaf and stem. The necrosis originates in the phloem groups, and spreads gradually to the surrounding tissues. The cells in the vicinity of the necrotic areas are stimulated to abnormal growth.

In leaf-roll the cells of young leaves which have not begun to roll appear normal. When rolling commences it is found that there is abnormal starch accumulation in certain groups of cells throughout the lamina. Finally, in definitely rolled leaves, the chloroplasts of every cell are bloated with starch, and in the petiole and stem abnormally large starch grains are also present. Since a hilum and striations are visible in these large grains, it is evident that translocation of starch does not take place as in the normal plant.

Abnormal inclusion bodies, similar to the bodies described by other workers on virus diseases, are found in the chlorotic areas of the leaves in association with simple mosaic, interveinal mosaic, crinkle, and streak; but were not seen in aucuba mosaic or leaf-roll plants. They do not occur in healthy leaves.

The X-bodies are most abundant in interveinal mosaic and crinkle plants which are suffering from the prolonged effects of disease. They are inconspicuous in young diseased plants.

The appearance of the X-bodies in living and in fixed material is described. Typically, the bodies are more or less rounded vacuolate masses situated in the cell cytoplasm close to the nucleus, and resembling protoplasm in appearance and staining properties. Mitochondria are present in the X-bodies, as in the cytoplasm of a normal cell. Numerous fatty globules are associated with the X-bodies at certain stages.

The cells in which X-bodies are present are considered to be in a state of comparatively low vitality, and in the later stages there is evidence of the breaking down of the lipoid-protein complex of the cytoplasm.

It is suggested that the X-bodies arise as a result of viscosity changes in the cytoplasm of the diseased cells, but the initial cause of these changes is unknown. The bodies, consequently, are regarded as an effect and not a cause of disease.

The fact that X-bodies are found in association with some virus diseases but not with others indicates their possible use in the clasification of these diseases.

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DESCRIPTION OF PLATES.

PLATE 4.

Drawn from living material.

FIG.

1. Vacuolate X-body adjacent to nucleus in living palisade cell. Interveinal mosaic, President. × 1200.
2. X-body in living cell of spongy mesophyll. Nucleus at upper edge of body. Crinkle, President. × 1050.
3. Vacuolate X-body, drawn out into a narrow process, in living palisade cell. Crinkle, Irish Chieftain. × 1050.
4. X-body in living palisade cell. Crinkle, Irish Chieftain. × 1200. There is a large fat globule in the cell in addition to the smaller fatty globules associated with the X-body.
5. X-body adjacent to nucleus in living epidermal cell of leaf. Interveinal mosaic, President. × 900.
6. Living parenchymatous cell from midrib of leaf, showing small protoplasmic mass which was being carried along in the cytoplasmic stream. Crinkle, President. × 900.
7. Elongated vacuolate body in living sub-epidermal cell of midrib of leaf. Crinkle, President. × 900.
8. Living hair cell, showing vacuolate X-body adjacent to nucleus. Crinkle, Irish Chieftain. × 500.
9. Living hair cell, showing vacuolate X-body adjacent to nucleus. Interveinal mosaic, President. × 500.

10. Living hair cell, showing striated masses. Crinkle, President. × 500.
11. Large vacuolate X-body in living hair cell. Crinkle, Irish Chieftain. × 500.
12. (*a*) Pulsating body, apparently surrounded by a membrane, seen in living hair cell. Crinkle, Irish Chieftain. × 1150.
    (*b*) Same, showing protuberance of membrane, leaving clear space between membrane and contents.

Plate 5.

Drawings from sections of leaves fixed in Regaud's fluid, and stained with iron alum hæmatoxylin, and erythrosin. Mag. 1500.

Fig.

13. X-body adjacent to nucleus in cell of spongy mesophyll. Simple mosaic, President.
14. Palisade cell from leaf of young plant, showing irregular vacuolate mass stretching across cell. Crinkle, President.
15. Another palisade cell from leaf of young diseased plant, showing more or less rounded X-body.
16. Typical, rounded X-bodies in palisade and spongy mesophyll cells of old diseased plant. Crinkle, President.
17. X-body, showing vague structures in vacuoles, in cell of spongy mesophyll. Interveinal mosaic, Majestic.
18. X-body in cell of spongy mesophyll from leaf of streak plant (President) which showed a mottle preceding necrosis.
19. X-body in cell of spongy mesophyll. Interveinal mosaic, President.
20. Typical X-body in parenchymatous cell adjacent to vein. Interveinal mosaic, President.
21. X-bodies of various sizes from cells of spongy mesophyll. *a*, *b*, *c* from leaf affected with interveinal mosaic; *d* and *e* crinkle.

Plate 6.

Drawings from sections of leaf of Majestic plants affected with interveinal mosaic. Regaud fixation.

Fig.

22. Mesophyll cell, showing X-body with elongated structures in the vacuoles. A broad band of protoplasmlike material, staining pink, like the X-body, is also present, but does not appear to be an extension of the body. It resembles a thickened cytoplasmic trabecule. Stained with hæmatoxylin and erythrosin. × 1600.

23. X-body partly covering nucleus. In the lighter staining cytoplasm, lying between the vacuolated mass and the cell-wall, are black-staining filiform structures. Stained with hæmatoxylin and erythrosin. × 1600.
24. Mesophyll cell, stained with acid fuchsin and methylene blue. Showing large X-body pressed against nucleus, and crystalloid structure, which is stained deep blue. Chloroplasts, nucleolus, and mitochondria are red. × 1570.
25. X-body apparently drawn out to form an appendage, in which is situated a deeply-staining crystalloid structure. Acid fuchsin and methylene blue. × 1570.
26. X-body of irregular shape in mesophyll cell, with black filiform structure, similar to those in Fig. 23. Stained with hæmatoxylin and erythrosin. × 1700.

PLATE 7.

FIG.

27. *a*. Large X-body, with numerous vacuoles, from palisade cell of leaf. Crinkle in Irish Chieftain. *b*. Same at different optical level. × 1150.
28. *a–c*. Three different views of degenerating X-body in mesophyll cell of leaf. Interveinal mosaic. A brownish mass, resembling a tannin ppt., occupies the centre of the body. × 1050.
29. *a, b*. Degenerating X-body in mesophyll cell, drawn at two different optical levels.
    c. View of same body in adjacent section. × 1150.
30. Very large, old body in palisade cell. Interveinal mosaic, Majestic. This body was of brownish colour and granular structure. × 1000.
31. X-body, vacuolated nucleus, and degenerate chloroplasts in palisade cell of leaf. Interveinal mosaic, Majestic. In the adjacent palisade cells the chloroplasts were comparatively normal. × 1000.
32. Spherical bodies, staining black with hæmatoxylin, in tannin-network beside X-body. See p. 158. × 1000.
33. Living palisade cell filled with large starch masses from leaf of President affected with aucuba mosaic. See p. 149. × 820.
34. (*a*) Chloroplasts of normal appearance from green area of leaf. Interveinal mosaic in President.
    (*b*) Chloroplasts from adjacent chlorotic area. They contain more starch and do not stain so deeply with hæmatoxylin × 1180.
35. (*a*) Group of chloroplasts of normal appearance from green area of leaf affected with aucuba mosaic. Regaud fixation and hæmatoxylin stain. Mitochondria are shown in the cytoplasm.
    (*b*) Chloroplasts from adjacent chlorotic area, containing abnormal quantities of starch, and less deeply stained than the plastids of the green area. (The lower ones are stained acid fuchsin and toluidine.) × 960.

37 & 38. Degenerating chloroplasts from yellow area of severely mottled aucuba mosaic leaf.

39. Groups of chloroplasts from cells of yellow area of leaf affected with aucuba mosaic. In the chloroplasts and surrounding cytoplasm are spherical granules larger than mitochondria. See p. 149.

Plate 8.

Photomicrographs from sections of leaves, fixed in Regaud's fluid, and stained with hæmatoxylin and erythrosin. Figs 1–7 from Majestic plant affected with interveinal mosaic.

Fig.

40 & 41. Typical rounded X-bodies in cells of mesophyll tissue. × 960.

42. X-body and deeply stained crystalloid structure in mesophyll cell. × 960.

43. Degenerating X-body, the central portion of which is occupied by a brownish mass, resembling a tannin precipitate. × 900.

44. Irregularly-shaped X-body in palisade cell. Two filiform structures, staining black with hæmatoxylin, are situated on the vacuolated mass. × 960.

45. Large, old body in palisade cell. This body was of brownish colour, and granular structure. × 900.

46. (*a*) Low-power photograph of green area of mottled leaf, showing the comparatively normal appearance of the cells.

(*b*) Photograph of adjacent chlorotic area in same section. The leaf is thinner in this region, and X-bodies are present in many of the cells. × 95.

47. Low-power photograph of section through leaf of President plant affected with streak. The abnormal size and shape of the cells in the vicinity of the necrotic areas are apparent. The chloroplasts in many cases have distintegrated, and the palisade cells in the necrotic region are filled with a fine tannin precipitate. × 95.

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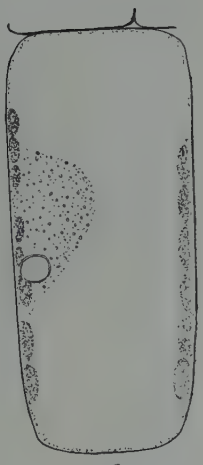
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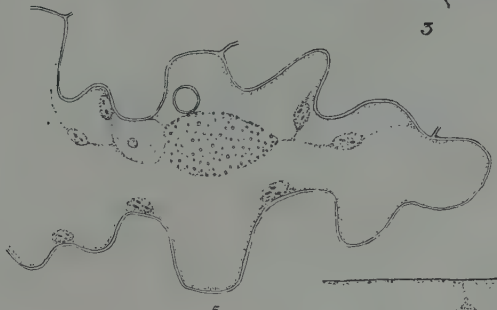
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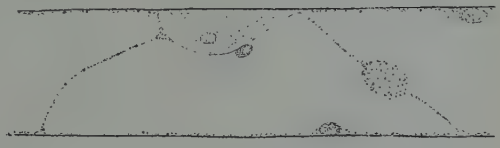
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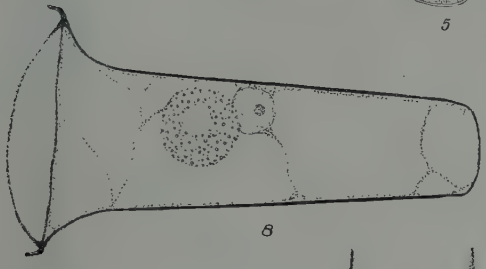
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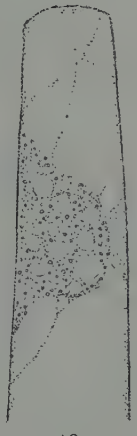
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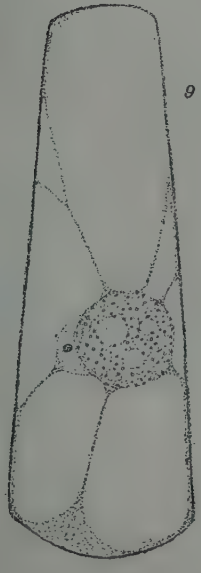
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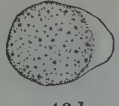
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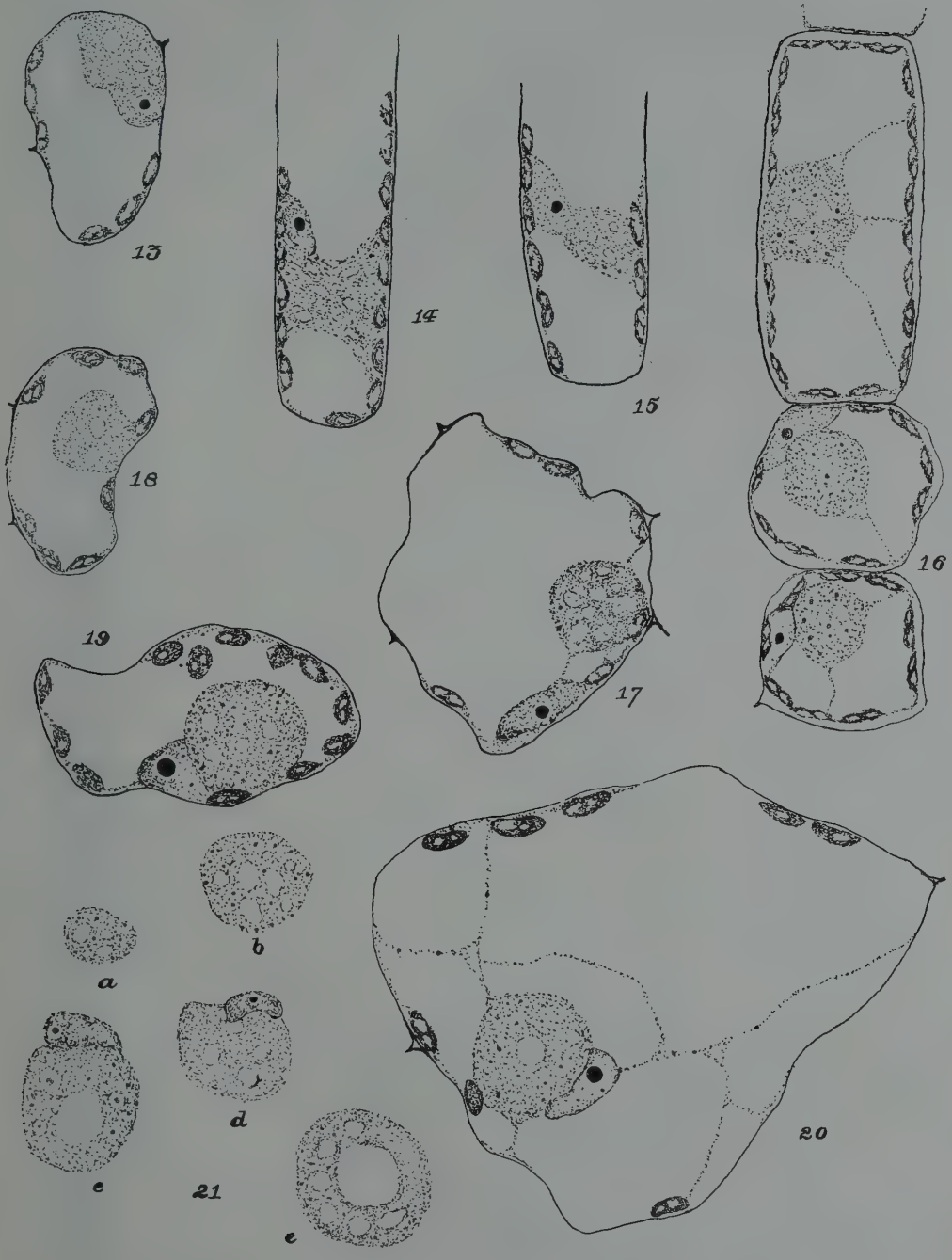
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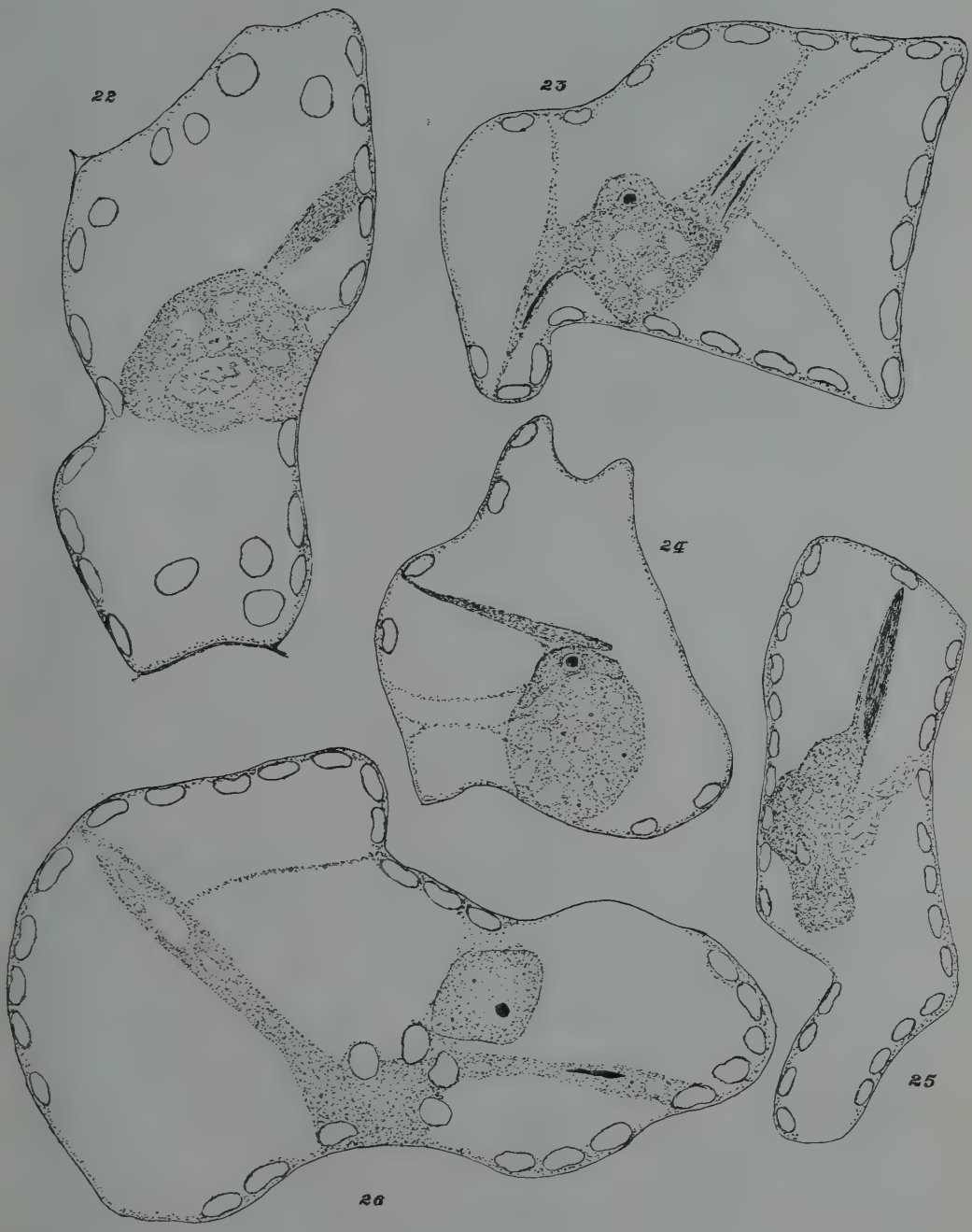
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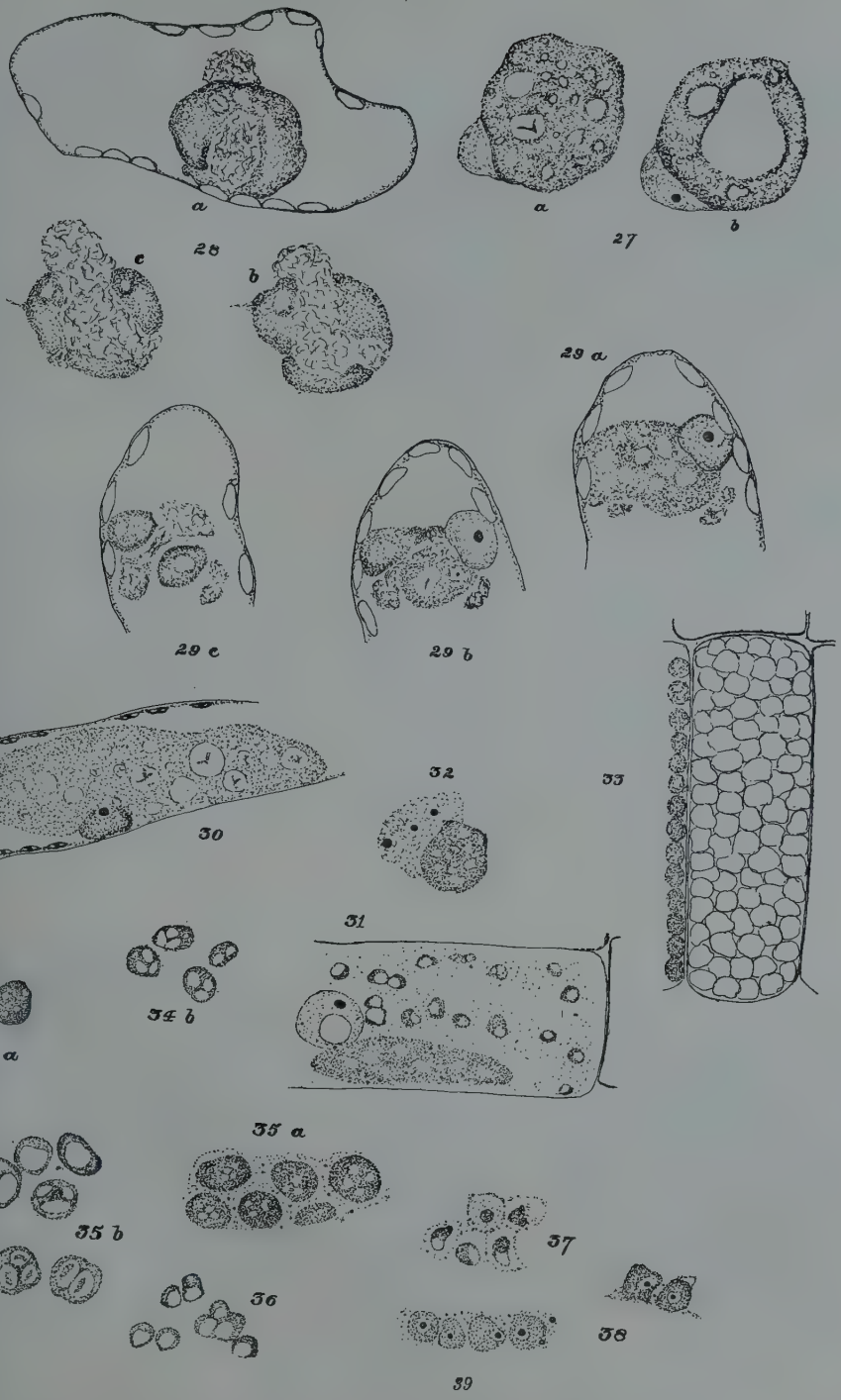
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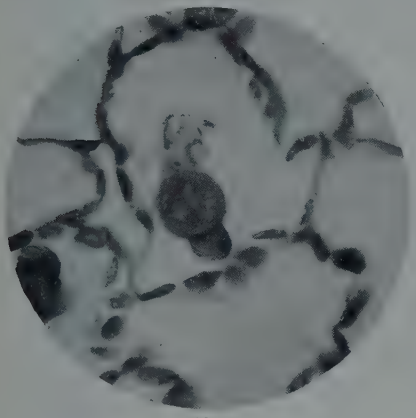

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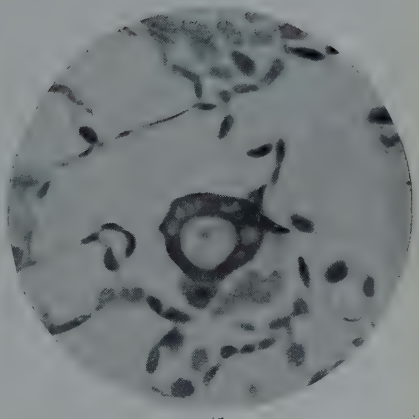

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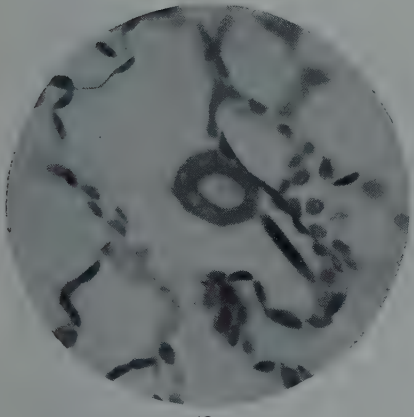

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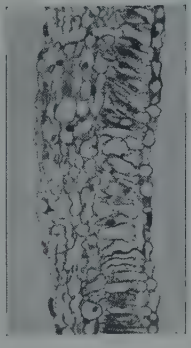

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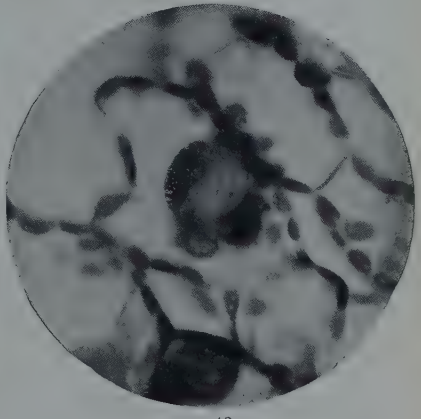

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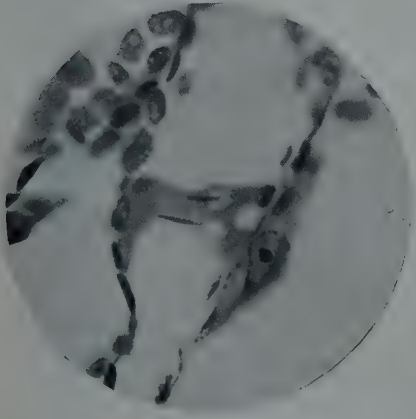

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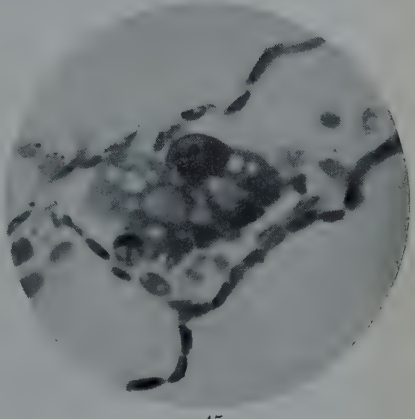

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No. 16.

THE EFFECT OF AN INSUFFICIENT SUPPLY OF VITAMIN D ON THE GROWTH OF THE SKELETON AND INTERNAL ORGANS OF CHICKENS.

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(Plate 9.)

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The incidence of rickets in chickens has received much attention, and the prevention and cure of the condition have been the subject of extensive study. The rachitic disorder may become evident at any time during the first half of the growing period, but it is most liable to occur about the third or fourth week of age. It is accompanied by very definite symptoms. The rate of growth slows down; the bird assumes a dull appearance; the gait gets unsteady; the legs fail to maintain their upright position, and bend inwards or outwards; the wings droop; and finally, the erect position is no longer possible, the bird ultimately lying on the floor in a characteristic awkward and painful position. The bones of the rickety specimen are much softer than normal bone, and the ash-content thereof is below normal. Thanks to the intensive campaign of Research stimulated by the discovery of vitamins in 1910, and to the phenomenal advance in the knowledge of these nutritive factors during the past two decades, definite information is now available on the incidence of rickets. The etiology of the rachitic condition known as leg weakness in chickens has been shown to be similar to that of cramp in pigs and rickets in dogs, rats, monkeys, and the human (1).

The rachitic disorder is due to the improper assimilation and utilisation of certain inorganic substances, particularly calcium and phosphorus. Provided the inorganic materials in question are supplied, their assimilation depends on the supply of vitamin D or the so-called anti-rachitic vitamin. Evidence from numerous sources shows that rickets may be prevented by exposing the chickens to sunlight, (2), (3), (4), (5), (6), (7), or to the artificial illumination from a mercury vapour lamp under certain conditions (5), (8), (9), (10), or by the consumption of irradiated ergosterol (12), (13), or potent cod-liver oil (4), (9), (11), (14), (15), (16), (17), (18,) (19), (20), (21), (22). Green foods such as grass also provide chickens with a limited supply of vitamin D (2), (9), (23).

Chickens which are seriously affected with rickets at an early age usually die unless the anti-rachitic vitamin is forthcoming. Even when the vitamin is supplied, where the affection on the previous vitamin-deficient condition has been very serious, death supervenes or a definitely abnormal limb development persists. Frequently, however, a recovery is made, and when the rachitic condition is so slight as to be, from the appearance of the limbs, only just apparent, a complete recovery seems to be effected. The question arises as to the effect, on body growth or subsequent productive powers, of a mild attack of rickets, which apparently passes off, or as to the effect of limiting the supply of vitamin D to such a quantity as would suffice to prevent obvious rickets, but which may probably be insufficient for all body requirements.

Experimental.

With a view to secure information on the subject, an experiment was conducted on chickens which were given sufficient vitamin D to tide them over the delicate young stage, and some of which were later subjected to vitamin D starvation. Sixty-four freshly hatched chicks were reared for ten days in a house to which direct light had access, and then divided into two groups of thirty-two each, both of which were transferred to houses lighted through window-glass. Each group contained an equal number of each of the following breeds:—white leghorns, white wyandottes, Rhode Island reds, and buff orpingtons. The chicks were fed from the beginning and throughout the entire experimental period on an all-mash diet of—

6	per cent.	Separated milk powder.
6	„	Low oil meat meal.
20	„	Bran.
30	„	Yellow maize.
40	„	Pollard.
½	„	Steamed bone flour.
½	„	Ground limestone.
½	„	Common salt.

In addition oyster shell grit was fed *ad lib.*, and water was before the birds at all times. The bedding after the tenth day was unchopped straw. Thus after the tenth day the conditions of rearing, both from the point of view of food and housing, precluded any intake of vitamin D, except what was experimentally added to the above basic diet. Roots for perching were not provided at any stage of the experiment. After the tenth day Group I received the basic diet only, while Group II got an addition of 1 per cent. of cod-liver oil, which had previously been shown to be potent for vitamin D.

At the age of ten days, *i.e.*, when the experiment proper began, the average weight of the chicks in each group was a little over three ounces, and the history of the two groups during the subsequent thirteen weeks of the experimental period was as follows:—

Group I.—After three weeks on the experimental treatment some of the chicks developed a stilted gait, which gradually grew more pronounced, and in the course of five weeks developed into lameness. Later on other chicks became lame, and by the end of the test quite a number of the individuals in the group showed the leg symptoms characteristic of rickets. Figure 1 indicates the condition of the group at the end of thirteen weeks of test. Some birds were definitely rickety; others showed fairly definite signs of the disorder; in others it was just perceptible; while others still appeared to be normal except for size, which was somewhat, but not very much, below that of the comrades in Group II.

Group II.—The birds in this group grew normally, and at no stage did they show any evidence of a rickety condition. Figure 2 indicates their appearance at the end of the period of treatment.

The weights of the two groups at the end of thirteen weeks' experimental treatment were as follows:—

	Average of Cockerels.	Pullets.
Group I	2 lb. 5 oz.	1 lb. 13 oz.
Group II	3 lb. 7 oz.	2 lb. 14 oz.

The food consumption per chick was more or less uniform throughout; but a very noticeable feature was the fact that Group I consumed more than twice as much oyster shell grit as did Group II.

An examination of the birds in the two groups at the end of the experiment revealed some interesting facts. Ninety per cent. of the chickens in Group I had crooked breasts—a disorder which developed in several chicks not showing leg weakness during the experiment, as well as in almost all those chickens whose limbs got "rickety." There was no trace of crooked breast in the birds of Group II. In Group I the bones of all the birds without exception were soft, in some cases so soft that a finger-nail could practically cut through them—a feature attributable, as a great volume of work on pigs, rats, and chicks shows, to a deficiency of "minerals" in the bone and a disproportionately large quantity of soft organic matter. When the skin was stripped from the body of some "rickety" specimens from Group I and from the corresponding birds of Group II the effect on body growth and chest measurement of the presence and absence of vitamin D became quite apparent—see figure 3. On further dissection a really wonderful difference in the development of the various body organs was seen, and this was best exemplified in the chest organs, particularly

the lungs, which in the "rickety" specimens were less than half the size of those of the normal bird. The pronounced "rickety" chest cavity was in all cases of miniature dimensions, while in cases of less pronounced rickets and of non-apparent rickets the capacity of the chest, though not so diminutive as in the previous group, was much less than in the normal bird. The remarkable difference in the development of the skeleton of "rickety" and "non-rickety" birds is shown in figure 4, in which attention is particularly drawn to the relative size of chest in the two specimens.

For eight weeks after the termination of the period of differential treatment a number of selected birds from each group was kept under observation on a common diet in the presence of direct light. The birds of Group II grew normally, while those of Group I, even the birds which had not previously developed leg weakness and typical rickets, were unable to take advantage of the improved conditions. They made very poor progress, and examination of the dissected bodies and skeleton at the end of eight weeks of sunshine treatment showed no improvement in chest capacity and very little increase in skeleton and body development, though the previously formed bone did harden to normal consistency.

Discussion.

The inability of the bird to utilize mineral matter as a result of vitamin D deficiency was clearly shown in this investigation. The bones of the birds which were deprived of the vitamin became soft and plastic, and chemical analyses showed them to contain very much less mineral matter, especially calcium and phosphate, than was contained in the bones of the birds of the other group. A deficiency of mineral matter in the food of chickens retards their growth very seriously, even in the presence of sunlight or other sources of ultra-violet rays (24), (25), (26). It is clear that in the absence of mineral matter the bird cannot possibly build a frame or develop normal organs, because synthesis of mineral matter from other food ingredients is impossible. Yet there is evidence of a complementary relationship between mineral intake and vitamin D. When the inorganic ingredients, particularly the calcium and phosphorus of the food, bear a certain optimum relationship to one another, the requirements of the anti-rachitic vitamin are at a minimum; and when, on the other hand, the chicks are flooded with ultra-violet light rays, the necessity for the maintenance of a definite relationship among the mineral ingredients is not quite so great (27), (28), (29), (30). It must be remembered, however, that the vitamins and minerals cannot replace one another in the body, and the complementary relationship does not provide for the absence of either from the nutritive menu. In the experiment herein reported the meals contained abundance of phosphorus, and the calcium was provided *ad libitum*; and in the case of Group I, which was deprived of the anti-rachitic factor, the consumption of calcium was twice

what it was in the other group. Yet the extra intake of calcium did not avail the bird in its effort to build up a normal body and healthy bone, the want of the other factor definitely limiting its capability to assimilate mineral material.

The lack of the food factor, which causes leg weakness or rickets in chickens, is responsible for very fundamental disorganization in the growth of the various body organs. Not alone is the bone not completely ossified—that is, hardened by the necessary deposition of calcium phosphate and other inorganic matter—but the quantity of skeletal material formed is less than it would be in normal circumstances. This is particularly noticeable in that portion of the skeleton which surrounds the chest organs. The bones in this portion of the body suffer in a similar way to the long bones of the limbs, which bend under the weight of the body. The weight of the body is partly thrown on the chest bones when a bird sits, and the effect is a distortion of the chest cavity out of its normal shape, and a reduction in its size. In consequence of the reduced size of the chest chamber the lungs of adolescent birds which have grown under conditions of vitamin D deficiency are very much below normal size. The heart, liver, kidneys, food tube, and reproductive organs are also smaller than the similar organs in normal individuals. Birds which suffer so severely as to exhibit signs of rickets during growth develop such a diminutive chest that they cannot possibly function usefully as productive units in after life even under optimum conditions. The size of the chest organs determines the quantity of blood, carrying the raw materials for manufacture, which reaches the various parts of the laboratory or factory of the body, and in the case of the horse, cow, pig, and other farm animals individuals of a comparatively small chest capacity give poor results in the way of work, milk production, or fattening capability. What holds in this respect for mammalian farm animals is equally applicable to fowl, and where the rachitic effect on the chest is of a severe order in early life the lungs become liable to disorders, which later become apparent in lack of vitality and frequently in the development of fatal lung diseases. While, however, a serious lack of the anti-rachitic vitamin produces results which are so decided in character as to be of obvious consequence, an insufficient supply may show no apparent results, but nevertheless be responsible for the production of chest and other body organs sub-normal both in size and efficiency. In short, the capability of a fowl to subsequently produce eggs to the limit of her inherited potentiality or to develop into a suitable carcass of meat is seriously affected by even a moderate degree of anti-rachitic vitamin deficiency during the growing period. In the case of the chicken reared for table purposes, not alone the size but the shape of the carcass is affected to a degree proportional with the degree of vitamin D shortage, and the crooked breast so frequently observable is definitely due to an insufficient supply of this nutritive factor. In connection with the production of crooked breasts in fowl interesting results are reported by Halpin and Holmes (31), who selected straight-breasted pullets of five months of age, and by supplying them with varying amounts of vitamin D for the subsequent seven months were able to demonstrate a connection between the

number of crooked breasts at the end of the experiment and the quantity of the vitamin provided during the experimental period.

Summary.

Evidence is brought forward to show that the anti-rachitic factor is, apart from its action in preventing leg weakness, of very great importance in body development, its absence resulting in soft bone, crooked breast, reduced size of skeleton and internal body organs, and particularly in a very serious distortion and reduction in size of the chest cavity and the organs thereof. A "rickety" chest remains undeveloped under subsequent favourable conditions, and, in consequence thereof, chickens which suffer even from a mild and non-apparent attack of rickets at an early age are definitely prevented from reaching the limit of their inherited capability either as fatteners or egg producers.

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FIGURE 1.—CHICKENS FROM GROUP I SHOWING A RICKETY CONDITION.

FIGURE 2.—HEALTHY CHICKENS FROM GROUP II.

(*a*) (*b*)

FIGURE 3.—BODY STRIPPED OF SKIN TO SHOW (*a*) NON-RICKETY, (*b*) RICKETY CONDITION. NOTE IN (*b*) DISTORTED AND REDUCED CHEST CAVITY, AND CROOKED BREAST BONE.

(*a*) (*b*)

FIGURE 4.—SKELETON OF NORMALLY DEVELOPED BIRD (*a*) COMPARED WITH THAT OF A SIMILAR BIRD (*b*) WHICH SUFFERED FROM A DEFICIENCY OF THE ANTI-RACHITIC VITAMIN.

No. 17.

REPORT ON THE RECENT BOG-FLOW AT GLENCULLIN, CO. MAYO.

By A. D. DELAP, A. FARRINGTON, R. LLOYD PRAEGER, and LOUIS B. SMYTH.

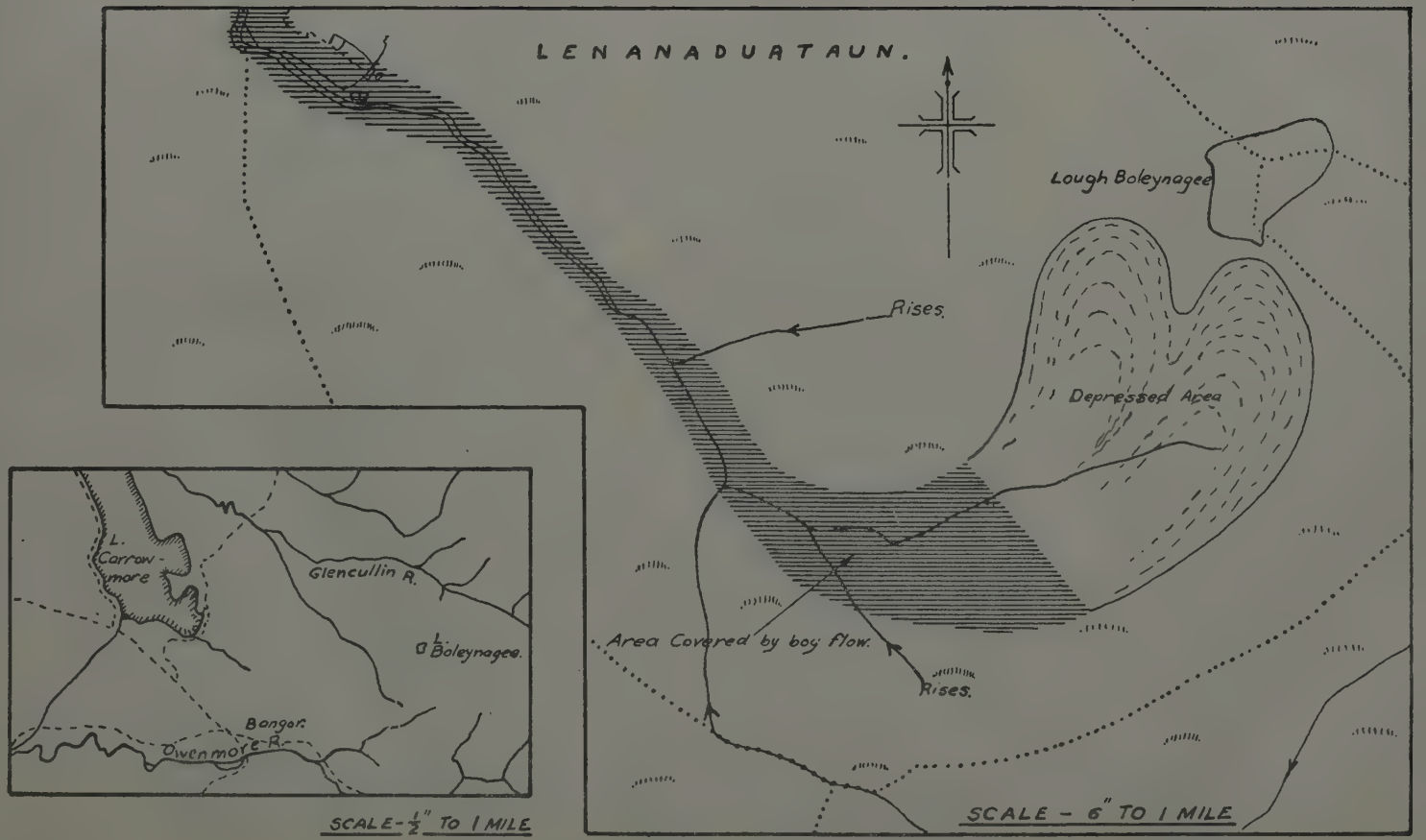
[Read November 24, 1931. Published separately April 15, 1932.]

Preliminary.

On Sunday, February 22nd, 1931, at about 10 a.m., an extensive bog-slide occurred in Co. Mayo, which did a considerable amount of damage, destroying one house and covering a large proportion of the scanty arable land along the valley down which it swept for a distance of about two and a half miles. As occurrences of this kind are rare and have a considerable scientific interest, as well as serious repercussions on the life of the affected area, investigation appeared desirable. An application to the Royal Dublin Society for funds to enable this to be done met with a prompt response. An excellent report from Mr. Flanagan, County Surveyor of Mayo, to the Land Commission, kindly placed at our disposal, gave us a succinct general account of the occurrence, and allowed us to make plans for its investigation. We went to Mallaranny on March 24th, devoted the whole of the next day to an examination of the area, and returned to Dublin on the 26th.

Description of the Locality.

The northern continuation of the Nephinbeg range forms an arc of flattish hills convex to the west and curving round towards Ballycastle. It rises to 1090 feet in Slieve Fyagh, to the east of Carrowmore Lake. South of Slieve Fyagh is an eminence, which attains 610 feet. From this hill a long spur runs north-west, separating the valley of the Glencullin River from that of its tributary, the Muingnakinkee, to the south-west. This latter stream is one of many draining a high-level bog which covers the ridges between the Glencullin River to the north-east and the Owenmore River to the south. The Muingnakinkee is normally a very small stream, and it winds backwards and forwards across a flat area between banks about 12 feet high, capped with bog. This flat, sometimes 50, sometimes 80 yards wide, is liable to flooding. Here and there there has been some cultivation between the stream and the bog, but on a very small scale.



BOG SLIDE - CO. MAYO - FEB. 1931.

There were only two inhabited houses along this stream above the by-road that serves the Glencullin valley, both close to the stream and between the bog edges, with small patches of cultivated land still closer to the stream. Just above the by-road there were also a few small cultivated patches, which belonged to one of the houses in the main valley.

On the ridge above the Muingnakinkee there lies a small shallow lake, Lough Boleynagee, some 300 yards long by 200 yards wide. It does not occur at the top of the rise, but rather on a slight flat which lies some 80 feet below the summit. As existing at present, there does not appear to be any real depression in which the lake is contained. On the down-hill side the bank of the lake is all peat, and a sounding taken to a hard bottom at the edge of the lake at this point, showing a depth of 12 feet of peat, makes it clear that portion of the lake, which is not more than a foot or so deep at this side, must overlie peat. On the other side of the lake the shores are of gravelly material, largely of local origin. The lake is, therefore, held up against the slope of the hill by a bank of peat. It is evident that the peat cannot have grown in this position, so that the present extent of the tarn must be the result of the enlargement of a small pan which occurred on the peaty hillside.

The six-inch map of the Ordnance Survey shows no stream flowing out of the tarn. Below it, to the south-west, a stream is marked rising at a distance of 570 yards from the tarn, and running directly away from it into the Muingnakinkee River. Its length was 430 yards. The slope of the undisturbed bog surface from the tarn down to the rising of the stream is about 3°, and from the rising to the Muingnakinkee about 4°. The gradient of the Muingnakinkee just below the junction with the small side stream is about 2°. Two miles below this point it joins the Glencullin River, its gradient having been reduced gradually to 1°.

The bog surface all around the area of flow is uniform in character—smooth and comparatively dry and hard. Its flora and other characteristics showed that this was not due to the dry weather prevailing at the time of our visit and for some weeks beforehand, but represented its usual condition. Ling (*Calluna vulgaris*) and the two common cotton-grasses (*Eriophorum angustifolium* and *E. vaginatum*) were the principal plants, with *Carex binervis*, lichens (e.g. *Cladonia*) and patches of rushes; *Sphagnaceae* were poorly represented. So far as could be judged by the eye, similar bog extended over wide areas on all sides—save where on a flat hill-top some bog pools glistened; and there was nothing in the surface indications—flora, slope, &c.—to suggest that the slipped area differed in any way from thousands of acres in the vicinity.

The depth of the bog at various points, as found by probing to solid bottom, was as follows: near the break in the slope at the rising of the stream, 9 feet; at the angle on the northern side of the subsided area, 10 feet; at the bottom of the channel that lets the water out of Lough Boleynagee, 8 feet; but as the spot sounded was 4 feet below the general level of the bog surface, the real depth of the peat at this point was 12 feet.

The Flow.

The flow commenced along a line a little more than half way between the tarn and the Muingnakinkee, where the slight break in the slope already mentioned occurred, the lower semi-liquid portion of the bog moving rapidly down the slope, bearing on its surface the lighter and tougher upper layer, broken up into a series of rafts. The debris poured down the slope and entered the Muingnakinkee valley almost at right angles. It surged up the other side, which here is steep, to a height of 22 feet 6 inches above the floor of the valley. The rush of the flow up the opposite side was at the time of our visit beautifully marked out in a curve in a vertical plane made by the residue of black under-peat and the rafts of the upper peat which had stranded. This feature, indicating the impetus of the flow, and its greater velocity along its middle line, has been also noticed in the case of the Kerry bog-flow of 28th December, 1896,[1] which at one point entered a river valley almost at right angles, as in this case. Immediately after the surge of the first rush, which carried the debris up the opposite slope, the semi-liquid mass began to flow to the right down the valley. This fact is well demonstrated by the furrows which have been torn by included snags in the grassy surface over which the mass swept. The scars made during the first rush are seen directed down the slope at right angles to the line of the main valley. These are crossed by a second set of striae, curving gracefully down the valley, which were formed when the local topography began to dominate the direction of flow.

Above the point where the initial burst occurred, the escape of the underlying semi-liquid bog let down the surface, which broke up into a series of great "floes" or "rafts." These floes were 5 or 6 feet wide, and of any length up to 20 feet or so, the crevasses between them being 4 to 8 feet wide, and filled nearly to the top with semi-liquid peat. The floes nearest the edges of the collapsed areas were very little disturbed, having simply dropped a foot or so; but as one passed from floe to floe towards the centre of the depression, the spaces between became wider, and the floes more irregular and tossed in all directions. At the centre of the depression the drop in level was from 12 to 15 feet. The torrent of liquid peat below the burst carried on its surface similar floes, and deposited lines of these, first on the surface of the undisturbed bog where this was overflowed, and then all along the sides and bottom of the valley of the Muingnakinkee. These stranded floes were seen to be more and more rolled and broken up according as their distance from the break increased. They were already rare at Glencullin Lower, though a few were still to be found even as far as the bridge which carries the main road from Bangor. The liquid torrent, besides the floes on its surface, swept masses of submerged and sodden peat along the bottom, with logs and roots of bog timber, and stones. It was not a quiet flow; there was every sign of great turbulence and great

[1] Sci. Proc. R. Dub. Soc. (N.S.), 8, 1897, p. 477.

violence, and one of those who heard it coming described the noise as like that of "fifty carts loaded with stones." A stone of half a hundredweight or more was noticed deposited at least 10 feet over the riverbed. As the torrent passed down the valley much material was deposited, first the lighter floating floes and the viscous black bog, which was usually left considerably deeper at the sides than at the centre of the flow, where the speed had been greater and where the stream had been at work clearing its course. The debris is usually confined to the bed of the river. When the flow of the liquid peat was at its height, it must have been at least 8 feet deep. A well-defined high-watermark is to be seen in places on the sides of the bed.

The fluid peat flowed out across the by-road which leads up the Glencullin valley, and filled the bed of the Glencullin River, flooding right across on to the other bank, and covering a certain amount of tillage land. It almost touched the wall surrounding a couple of houses (Martin Mullarkey's and his brother's). At the lower part of the Muingnakinkee valley the peat also flooded over some tillage land, and in both places covered some potato pits. The flow had already, at the time of our visit, been dug through, and some of the potatoes reclaimed and repitted. A very noticeable feature is the definite margin of the flow. It ends quite abruptly in a bank about 18 inches or 2 feet in height.

Statements by Local People.

Moran, the man whose house was destroyed, said that he first saw the flood when it was rounding a bend of the valley about two hundred yards above his house. It was at this time well confined within the valley, and he described it as coming "like a wall." He had just time to carry an invalid sister to safety and to loose his cattle before the flood lapped over the valley wall and struck the cottage. The cattle did not escape. He lost seven head and a donkey. Moran pointed out the place where the bog is said to have "burst." It is the place where the six-inch map shows the small side stream rising. His information on this point is based on statements of people from Upper Glencullin who were passing over the ridge at the time, and who saw the first breaking of the bog. According to some of the residents in Lower Glencullin, the flow, which started somewhere between 9 and 10 a.m., continued all day, and had practically ceased at nightfall.

Martin Mullarkey, who lives about two miles from the burst, compared the sound to that of an aeroplane. Winter, who was about a mile and a half from the burst, described the sound as being "like fifty 'buses." Moran compared it to a big 'bus or lorry. He also said that the advancing flood was covered as by a cloud or mist, and that lights were playing over it. Apparently in all cases they were describing the sound of the flood. There was no mention of anything in the nature of an explosion.

Effects of the Flow.

The shallow tarn, Lough Boleynagee, just above the head of the collapsed area, has dropped in level by 6 to 12 inches. This is shown by the abundant growth of the Water Lobelia (*Lobelia Dortmanna*), which normally grows submerged, but which now extends over the new foreshore to a height of about 6 inches above the present water-level. This subsidence has been caused by the removal of some slight obstruction beyond the present natural trench in the peat through which the lake drains, as the water still flows out over the bottom of the trench to a depth of several inches.

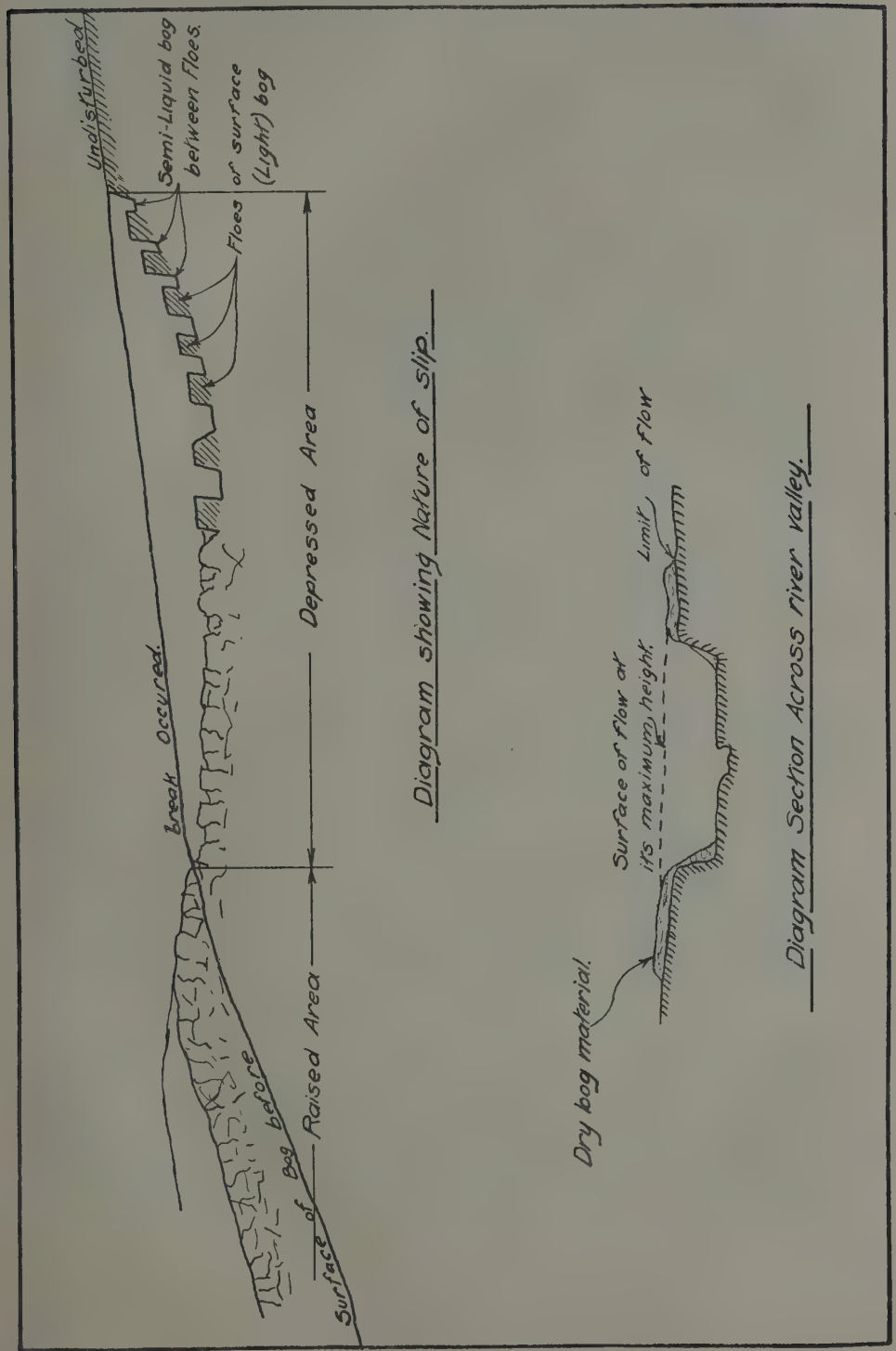
In what follows "Section 1" means the area which has subsided as a result of the flow; "Section 2," the path of the bog-flow as far as the Muingnakinkee; "Section 3," the narrower part of the valley of that river which the flow filled from side to side; "Section 4," the wide lower part, where the flow no longer filled it.

Section 1.—

This is an irregularly oval area, measuring about 560 yards down the slope and 400 yards across. Its upper limit is only about 7 or 8 yards from the edge of the tarn. The upper and lateral margins of this area are marked by scarps 2 or 3 feet in height, where the upper layer of the bog has broken and sunk, owing to the outflowing of the lower layer. Farther in from the margin the cracks become less regular, often recalling glacier crevasses on a small scale; and as the centre of the section is approached, the upper layer is more and more disturbed, being completely carried away over considerable areas.

The bottom of the fissures is occupied by the black under-peat, the surface of which now lies at about 3 feet below the surface of the upper peat, which extends between the fissures. The black peat seems to have been squeezed up within the fissures. In a wide fissure the bottom is usually somewhat higher in the middle than at the sides. This is probably a late feature due to the subsidence into the softer material of the rafts of upper peat. The sections of the upper layer of the bog which lie between the fissures appear on the hillside like the rows of seats of a theatre. Each is, however, tilted, so that the downhill side is somewhat raised. At the same time, the downhill face shows clearly that the under surface has been dragged forward by the material which flowed out from beneath. As we approach the upper limit of the disturbed area, we find that the fissures are less and less broad, and the moved portions of the upper surface have travelled to a much less distance. At the actual edges of the area of subsidence the surface peat has been torn away, but usually the end of each strip is still fixed to the undisturbed peat, so that the fissures form curved areas, narrowing to a point.

All these facts go to show that the major part of the disturbance has been caused by the fact that the under portion of the bog has flowed freely. This has carried with it a good deal of the surface of the bog, but this is merely an *effect* of the flow.


Undisturbed
Semi-Liquid bog between Floes.
Floes or surface (Light) bog
Break occured.
Surface of Bog before
Raised Area
Depressed Area
Diagram showing Nature of slip.
Dry bog material
Surface of Flow at its maximum height.
Limit of Flow
Diagram Section Across river valley.

Towards the centre of the depression the upper layer is completely divided into separate fragments. Much of this part of the area is now occupied by the remains of the black lower peat, but this is partly covered in places by the isolated fragments of the upper layer, which become more plentiful towards the margins. A stream now draining the tarn passes through this section, and continues through Section 2.

Section 2.—

This coincides roughly with the shallow valley of the tiny tributary to the Muingnakinkee, already referred to. But the bog-flow was wider than the trough, and the margins of the flow are now represented by tumultuous masses, up to 10 feet in diameter, of the upper portion of the bog stranded outside the limits of the depression, along with much of the soft under layer.

The original bog surface, with its covering of vegetation, was overwhelmed by debris, but is quite undisturbed. There are no grounds at all for the opinion which was locally expressed that the surface had been burst up by the slide penetrating and lifting it. The intact surface can be seen in many places, and by probing can be found everywhere at a depth of not more than 4 feet.

At the junction of Section 1 with Section 2, where apparently the moving bog had first mounted upon firm bog, the pile of "floes" on both sides was much higher, reaching to 8 or 10 feet, and forming one of the most striking phenomena connected with the occurrence. Between the two walls of jetsam was a sea of soft peat, about 200 yards wide, with "floes" scattered over it irregularly. Just above the region of the break there were large areas completely stripped of surface peat, and also two long islands of undisturbed bog. Rafts of surface peat have been heaped on top of these. In one place, at the northern end of the disturbed area, and just at the extremity of the line of break, a large raft has been rotated, so that the downhill portion has been pushed up to a height of over 4 feet over the undisturbed surface to one side, while the rest of it has sunk below the original surface to a depth of a couple of feet. The thickness of the raft is 4 feet.

The little tributary of the Muingnakinkee still makes its way down, partly on its original bed, partly deflected by, and flowing over, the remains of the bog-flow. It no longer "rises" at the upper end of Section 2, but is seen to proceed from the tarn and flow on the surface through Section 1.

Section 3.—

In this section the liquid peat was flowing rapidly between the walls of the valley of the Muingnakinkee, reaching a maximum depth of 12 to 15 feet. The greater part of the material left behind by the bog-flow consists of the soft under layer, but "floes" also occur. At first these are right side up, as in Sections 1 and 2, but lower down overturned sods become frequent, and in the

lowest mile of the section the sparse sods are rounded and worn, as if they had been rolled over and over. Along considerable stretches of this section the bog-flow overlapped the valley rim, and left a "lateral moraine" of the soft material on the surface. Wherever a gully entered the valley, the material had been forced up it for some distance. All along, wherever the flow did not overlap the valley rim, the top level is indicated by the black mark left on the white sandy faces of the meander scars.

The river had, as a rule, found its own bed again at the time of our visit, but in some places it was evidently deflected by the remaining peat of the flow. In this section, about quarter of a mile from the top, Moran's cottage and cow-house stood in a bog-cutting a little above the level of the stream. The bog-flow had approached these from the front, pushed in the front walls and pushed out the back walls, filling the buildings up to the eaves. One of the gable ends was upset, and could be seen lying in a sloping position in the peat, the thatched roof had collapsed, and corners of furniture and the snouts of two animals could be seen projecting above the surface.

Section 4.—

Here the valley of the Muingnakinkee opens out into a wide flat, confluent with that of the Glencullin River. A by-road crosses it, running parallel to the Glencullin River, in a west-north-west direction, to join the main road. On this flat, and crossing the road obliquely, lies a considerable deposit of the soft peat in a belt about 200 yards wide and 2 to 3 feet deep. Its margins run irregularly over the flat fields, and may be seen lapping round the mounds of potato-pits.

A rough estimate of the amount of material which had moved gave 400,000 cubic yards from the area above the point of bursting, and a similarly rough estimate of the amount deposited below the burst came to 320,000 cubic yards. The true figure probably lies between these limits.

The damage done is not easy to measure; the value of the grass land or the cultivated land covered by the bog-flow, if measured in acres and by what it would let for, would be very small, but to those who lived there it was all they had. Moran, whose house lay highest up the valley and nearest the burst, lost his house and practically all the land that could be cultivated, and all his few head of stock. Winter, the only other inhabitant of the valley above the road, was better off; his house was untouched, and some of his land lay high enough to escape the flow, but much of his land was covered. Other land, both above and below the road, will in time recover, but for this year, and probably some years to come, no crops can be planted and no grass will grow, and the very small margin between just living and starvation, which must be the usual condition in these valleys, will be very seriously reduced.

Outside the area actually covered no damage whatever had been done, except that the by-road has been cut through by the stream. Very little water seems to have flowed out of the mass, which is now rapidly drying. There is

no reason to believe that permanent damage has been done to the land, for in a very short time the peat which constitutes the flow will have dried enough to be cut, and after it has been cut the tillage land will be usable again. Indeed, at one place the edge of the peat has been cut back a foot or so to enable a furrow to be run straight. The reclamation of the land has already begun.

Causes of the Bog-flow.

It is not easy to account for the bursting of this particular area of bog; there was no face exposed leaving a point of weakness, as in the case of the Kerry bog-burst of 1896; there was no great change of gradient either on the surface of the bog or revealed by the burst, nor was there any considerable spring.

As has been mentioned earlier, there was a slight, though quite definite, change in the gradient of the surface on which the bog lies, about half-way between the small lake and the head of the Muingnakinkee valley. It was at this point that the initial burst occurred. The fracture was, probably, across the line of normal drainage indicated by the streamlet marked on the map, and may have been only some 50 yards long. It is clear that the condition of the under-peat above and below the line of fracture must have been different, since in the former area it flowed readily, while it still remained firm in the latter area. There was probably some tension in the upper stratum of the peat at the point where this change occurred. The drying of the upper layer of the bog by drought while the substratum was kept in a liquid condition by the drainage from Lake Boleynagee would tend to increase this tension to the breaking point.

The lower layer of the peat in bogs such as the one under consideration is absolutely without coherence. In this condition it has some properties in common with clay. Like the latter, it is impervious, that is to say, it offers great resistance to the flow of water. At the same time, like clay, it is capable of holding much water in its interstices, and, under certain conditions, this water may be present in such large proportion that the mass is better described as "water with peat in suspension" than as "peat with absorbed water." The difference in properties might be roughly compared to that between a sol and a gel. From the description given above, it is obvious that the material which flowed was in the gorged or "sol" condition.

Two questions then arise: first, why did this area of bog accumulate a surfeit of water at this particular time, and, second, what was the immediate cause of "bursting"? If we could get the answer to the first question, the second might not arise—the "bursting" may have occurred as soon as the accumulation of water had reached the critical point. Unfortunately we cannot solve either point with certainty.

The most suggestive feature is the relation between the tarn, the area of origin of the flow, and the source of the little side stream. It may safely be

assumed that the stream was to a great extent fed by the tarn, and that the connection between them was immediately under the peat. The stream is now visibly flowing from the tarn.

The lake as it is now constitutes no danger whatever, in spite of local opinion, as all the slipping which is likely to occur on this part of the hillside is already complete. The waters of the lake themselves are of very little consequence, as it is very shallow, and the quantity would be insufficient to cause much damage, even if let go all together. There is not enough to do more than help to clear away the debris deposited in the bed of the stream. Nor was it directly responsible for the flood and damage, as the amount of water which escaped from it would not have been sufficient to do harm. Its importance was in acting as a reservoir which kept the peat thoroughly soaked.

From the observations recorded above it may be taken that before the occurrence the stream in Section 2 flowed in a slight hollow, and that it emerged from under the peat of Section 1. There would, therefore, be a peat front overlooking the rising of the stream. If the lower layer of the peat reached the critical point as regards the proportion of water to solid, the hydrostatic pressure coming into play as a consequence would be most likely to cause a rupture of the fibrous upper layer just at this point. The hearsay evidence and the observation of the present conditions are in accord with this suggestion.

It was reported to us that people from Upper Glencullin who were crossing the hill at the time actually witnessed the burst. Their description was that the bog gave way just at the point where the spring rose; then the disturbance spread rapidly back up the hill and outwards. This agrees well with the shape of the disturbed area. The narrowest part is just where the burst is said to have occurred. Above this the fissured area widens out. Once rupture had occurred, all the gorged peat above this point would begin to flow downwards. The bottle-neck where the bursting occurred causing congestion, the moving mass would tend to mount the bog on either side, thus giving rise to the exceptional height of the lateral piles of "floes" in this vicinity.

There appears to be no evidence pointing to a cause for the bursting having taken place at the particular time at which it did. Perhaps there was an exceptional amount of water present owing to the very wet summer preceding. A very long period is probably required for the peat to respond to abnormal rainfall, owing to its impervious character. And also this area may have been ready to flow for years past, and the erosion at the head of the stream may have only now reduced the restraining influence to breaking point.

In the case of certain bog-flows the cutting of peat for fuel has been pointed to as a cause which released the pent-up material. In this case, as far as we could gather, there had been no cutting so far up the mountain side.

Again, strong storms blowing down the slope have probably been the immediate cause of movement in certain instances. Here, the peasants are unanimous in declaring that the preceding night, and the morning of the occurrence, were fine and dry, with no strong wind.

In the Kerry flow of 1896 a fault underlay the area of origin. It was suggested that movements along this fault, perhaps due to the Hereford earthquake, might have caused alterations of drainage whereby there was increased flow from springs under the bog. In the present case, though a fault passes across the valley lower down, there is no evidence of one under the area of origin or higher up the slope.

Although bog-flows are rare phenomena, a good many have been recorded, and some examined in detail. An account of those up to 1896, so far as ascertained, is given in the Report on the fatal Kerry flow of December 28th, 1896, in the Scientific Proceedings of this Society (**8**, N.S., No. 57)[2]; it would appear that they are essentially an Irish phenomenon. In most cases little damage has ensued, though in some cases bridges and houses have been swept away, and much land covered by a deposit of fine peat. This peat is eventually cut for fuel, and the land recovered. Lake and river fish have sometimes perished in large numbers from suffocation. An account of the site of a bog-flow after seven years will be found in the "Irish Naturalist," 1897, p. 201.

In September, 1931, one of the authors of the above report visited the site of the Kerry bog-flow of December, 1896. After 35 years the condition of this bog showed little trace of the catastrophe. The surface of the peat was not noticeably irregular, nor was there any trace of the flow downstream. The land just below the bog, which had been covered after the flow, was in cultivation, and had been for years. However, in places the cutting of peat for fuel in recent times had been extended into the area from which the old flow had proceeded, and it was possible to see that no depth of peat remained, the black under-peat being almost entirely absent. A large rushy area in the middle of the bog, where no peat is cut, presumably because as a consequence of the flow it no longer exists there in economic quantities, is now the only feature of the bog that can definitely be connected with the burst.

[2] See also *Irish Naturalist*, **6** (1897), p. 224.

No. 18.

A CRITICAL REVIEW OF SOME RECENT WORK ON THE OCCURRENCE OF VIRUS COMPLEXES IN THE POTATO.

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The independent work of Salaman[1] (7), K. M. Smith (11 and 12), and Murphy and M'Kay (5 and 6) all points to the conclusion that some of the naturally occurring potato virus diseases are complexes, or at least allows this as a possible conclusion. It may be said to have been established by analysis in the case of simple mosaic by Smith, and by synthesis in the case of crinkle by Murphy and M'Kay. Very similar results have also been recorded recently by Valleau and Johnson (13) and Koch (4), working independently in the United States. This indicates that the problems presented in the two continents are closely related, but on account of the obscure relationship still existing between the European and American potato virus diseases, it is not possible to deal adequately with the latter in this paper.

The discovery of the X and Y constituents associated with certain potato mosaic diseases by K. M. Smith opens up many new lines of work, and is considered to be of fundamental importance. One of the principal functions of this paper, however, is to endeavour to show that the finding of what are held to be the same materially unchanged X and Y viruses in association with diverse potato mosaic diseases is capable of more than one explanation, and does not necessarily connote the fact that these viruses are the sole or principal elements which underlie the diseases in question, or that the diseases are as closely related as such a composition has been held to imply.

The question has been raised as to the necessity for continuing the use of the terms crinkle, mosaic, and streak, since it is held that these diseases are

[1] It is unfortunately impossible to deal with Salaman's later work on the analysis and synthesis of virus complexes (*Rept. 2me. Congrès Internat. de Path. Comparée*, pp. 451–454, 1931). All our work on the crinkle complex, as well as this paper on the general question of virus complexes in the potato, which is the outcome of it, was substantially completed before the above preliminary report became available. In it Salaman's results are necessarily given in a highly condensed form, and to endeavour to fit them into any connected scheme would probably only lead to further confusion. For instance, a new virus Z has been found in crinkle, but no data are given on which a comparison can be based with the virus A which Murphy and M'Kay (6) have shown to be a constituent of crinkle. Under these circumstances it is preferred to wait for the full account of the work; but while no opinion can at present be expressed concerning the experimental results, attention may be drawn to the remarkable similarity of some of the general ideas concerning the association and dissociation of the elements of virus complexes to those contained in this paper.

complexes, and do not indeed differ fundamentally from each other(12). For those who are familiar with the economic problems involved, this question is easily answered. It will be necessary to continue to make use of them as long as these diseases or complexes occur and spread as such in potato crops, and are the cause, as is the case in these countries at least, of a large part of the injury associated with the general mosaic group. No evidence has been presented up to the present to show that any single virus is responsible for this injury to the exclusion of the complexes. The virus Y occurs alone in nature (12), but no record is given of the finding of X alone in a plant under natural conditions. As Salaman (7) says, some of these complexes are marked by such constancy of symptoms as to suggest a unit and specific virus, or at least a balanced and uniform mixture. It will obviously be as necessary to have names for the commonly occurring virus complexes as for common substances which are chemical compounds. The appearance and effects produced by such a complex are not necessarily those produced by its constituents, for it could not be foreseen that the combination of two diseases resembling simple mosaic in President would produce crinkle, any more than the physical properties of water could be deduced from those of hydrogen and oxygen.

As is indicated in another portion of this paper (p. 200), and will be more fully shown elsewhere, the economic effects of simple and interveinal mosaic, crinkle, and streak vary greatly in importance, depending on the variety of potato attacked. Streak is fatal to some varieties, but on others it produces no results of measurable practical importance. Crinkle is fatal to another group of varieties, but produces a varied series of effects on the remainder. Similarly the milder mosaics assume lethal characteristics on a third group of potato varieties, while usually being of the least direct importance. In this respect these diseases or complexes give the impression of a strong individuality rather than a very close relationship. From the practical point of view they must continue to be treated of as separate diseases, no matter what their constitution or relationship is ultimately shown to be. The terms, crinkle, mosaic, and streak, may be open to objection, but they have attained such currency and are so necessary to designate commonly occurring maladies that it would be inconvenient to replace them unless they are rendered so debased or generalized as to become useless, through being employed in a loose sense.

Comparison of virus Y from Up-to-Date streak with Y from a mild mosaic in Arran Victory.

The idea is developed in K. M. Smith's (12) latest work that most potato virus diseases are of dual nature,[2] and that their constituents are of the same nature throughout, namely, the Y virus, which is stable, and the X virus, which

[2] In addition to this statement, it is mentioned that there may be a third virus in crinkle and streak.

is variable. He says: "In the writer's opinion, the different symptoms exhibited by the various potato mosaic diseases are due rather to strains of the X virus differing in virulence rather than to distinct viruses. Thus it will probably be found that these two viruses, X and Y, alone or in combination, are responsible for the majority, though not all, of potato mosaic diseases." The present writer believes that this is a mis-reading of the evidence, and that there are alternative and preferable explanations of the experimental data, which are more in accord with evidence available from other sources. It is necessary to point this out in order to prevent further confusion, which would be fatal, for work on the potato virus diseases is already sufficiently difficult to understand, even for workers in this field.

In the first place, the conclusion that the Y virus occurs in the same unchanged condition in a form of mild mosaic, a form of crinkle in Myatt's Ashleaf and in Up-to-Date streak is in conflict with the experimental evidence, as given by K. M. Smith. The characteristics of this virus, originating from Up-to-Date streak (12), include (among others) the following:—

1. It is transmitted selectively by *Myzus persicae,* which in no case among the twenty examples of potato diseases studied has transmitted the whole complex to tobacco, potato, or any other plant.
2. It causes the disease in tobacco characterised by the development of a dark green colour along the veins.
3. After passage through tobacco, it causes in President potato a disease with (primary) symptoms of "leaf-drop streak and crinkle."

The corresponding virus previously isolated from a mild mosaic in Arran Victory and President, and subsequently identified as virus Y (11), appears to have radically different properties. It agreed with Y from Up-to-Date streak in being apparently selectively transmitted by *Myzus persicae* and in producing similar symptoms in tobacco; but when transferred from tobacco to President potato, it caused an intensified mosaic, without necrotic lesions. This result was secured in two series of experiments separately recorded in 1929 (8 and 9). Whatever tendency to necrotic lesions existed in this case was associated with the (X + Y) mixture, and not with the aphis-borne Y virus.[3] It is stated (9): "It would appear, therefore, that the aphis does not transmit some element of the virus which is concerned with the production of the more necrotic and lethal symptoms."

[3] When virulent ringspot of tobacco was returned to potato, a similar distinction held good: The (X + Y) virus produced "intensified mosaic + death," and virus Y "intensified mosaic – death," or, in other words, "the lethal character of the disease is not transmitted by the aphis" (this being virus Y), while it accompanies the needle-borne (X + Y) combination (9).

The Y virus similarly isolated from "crinkle in Myatt's Ashleaf" (10) agrees with the Y subsequently isolated from Up-to-Date streak (12) in producing severe necrosis when returned to potato from tobacco. It therefore differs from the Y virus as isolated from mild mosaic, although it is specifically identified with the latter by Smith (12).

TABLE I.—Characteristics of virus Y from different sources, as determined by K. M. Smith.

Source of virus Y.	Symptoms on tobacco.	Symptoms on President potato *ex* tobacco.
A mild mosaic in Arran Victory.	Vein-banding.	Intensified mosaic without necrosis.
A "crinkle" in Myatt's Ashleaf.	Vein-banding.	"Crinkle and leaf-drop streak" with severe necrotic lesions.
A streak in Up-to-Date.	Vein-banding.	"Crinkle and leaf-drop streak" with severe necrotic lesions.

It is probable that the basis on which this identification rests is to be found in the similar reactions of tobacco to the Y viruses from the three sources. As reference to Table I shows, the symptoms produced by all of them on this plant are apparently the same. On the other hand, the subsequent effects on potato, judging by the descriptions given, do not correspond in all cases. In view of this, it appears to be impossible to escape from the conclusion that the virus originating from the mild mosaic is not identical with the corresponding viruses from the streak and the crinkle. Whether the two last are themselves identical or not remains for the present an open question. While the classification of virus diseases depends for the most part on symptoms, it is necessary, before any given viruses can be identified, that the reaction of *all* the test plants employed in comparing them should be similar.

In Smith's later work the identification is implied of all "streak"-producing elements in all the potato mosaic diseases studied with the aphis-borne Y virus, although the obligatory relationship of Y and "streak" is in contradiction to this author's earlier work, as has been shown above. It is also to be observed in this connection that the question remains to be solved as to how there can be an exclusive and selective relationship between *Myzus persicae* and the Y virus (12), when this aphis has been found to transmit, apparently unchanged, a virus disease, such as interveinal mosaic, in which evidence was presented of the presence of the X virus as well, presumably, as that of Y (10).

It is essential for the purpose of suggesting an explanation that this discrepancy should be clearly stated. Its explanation may lie either in the fact that the viruses designated Y from a form of mild mosaic in Arran Victory and Up-to-Date streak, respectively, are entirely dissimilar, or that while they

contain a common element, one or other is itself a complex. As will be seen, reasons are given for the view that the Y virus from Up-to-Date streak carries with it another virus which does not alter the clinical picture of Y on tobacco. It is only to be expected, perhaps, that no single plant will give such precise and constant reactions as to distinguish all the potato viruses, but it is interesting to observe this phenomenon in the case of tobacco, as well as in some potato varieties.

It is known that streak in Up-to-Date is generally (but, as it is believed, not always) associated with a simple mosaic, which is also carried almost or entirely without symptoms. Up to the present no stock of streak in Up-to-Date has been found by the writer which is free from this mosaic. The effect of inoculating such a combination (streak + a simple mosaic) into tobacco would be, following Smith's work, to produce by appropriate methods symptoms of the X and Y viruses, respectively. So far as is known, the effect of pure streak on tobacco has not been determined, except that Smith (10) notes that an almost lethal potato disease, which is identified with the streak element of Up-to-Date streak, may be carried by tobacco almost without symptoms. If this streak produces practically no symptoms on tobacco, and if it were insect-borne to that plant, it would probably exist in tobacco side by side with the Y virosis, without altering the appearance of the latter; but its presence would be made evident when the impure Y (Y + streak) was inoculated into President. Hence, while Y from simple mosaic was marked by the absence of necrosis, Y from "streak" was found to be highly necrotic.

This amounts to the suggestion that the analysis in this case is not primarily concerned with streak, but with a mosaic present in addition to the streak, from which the X and Y are in part, at least, derived. To continue the algebraic notation, it is concerned with X + Y + Z,[4] and the characteristics given for Y are probably those of Y + Z. This conclusion is strengthened by the statement that Up-to-Date streak does not invariably contain two viruses (12), which would point to one of the viruses, when present, being an accident. In view of all that is known of the usual condition of streak in Up-to-Date, it would be necessary to prove the absence of mosaic before it can be accepted that the X and Y viruses derive exclusively from the streak element.

Comparison of virus Y from "crinkle" in Myatt's Ashleaf and Y from Up-to-Date streak.

A similar explanation is offered for the fact that the virus Y from the form of crinkle in Myatt's Ashleaf used by K. M. Smith (10) had a more severe effect on President potato than Y from a mild mosaic in Arran Victory. Either this "crinkle" contained no element common to simple mosaic, thus

[4] This was written before the description by Salaman of a new virus, to which the specific name Z has been given. The term is used merely for a hypothetical virus above.

giving an entirely different Y, or, as is more likely, it contained a common element, and also another virus which was carried with Y, and modified the resulting reaction on potato. According to my interpretation of the constitution of (standard) crinkle, simple mosaic is present in this disease (6), which would provide a common element. As in the case of streak, the results could be most easily explained by assuming the presence of another factor, B, in addition to X and Y. Neglecting X, with which we are not concerned here, Y should be equivalent to Y from simple mosaic, and B, if carried (or in part carried) with Y, would account for the altered symptoms of Y on President. It is suggested that the "crinkle" used by Smith was probably impure, which would make it possible that B itself was a complex, including probably the Irish Chieftain virus, to which the name A has been given (6), and an unknown element.

The "crinkle" material used by Smith unfortunately does not appear to have been standard crinkle, and its exact composition cannot be determined. Evidence is available to show that it can hardly have been fully identical with the crinkle used by ourselves and Salaman. According to Salaman (7), standard crinkle, when passed through tobacco, produces only a mild mosaic on potato, and no general necrosis or "streak." If this is so, and the fact is not doubted, it disposes of two important questions at once, namely, that Smith's "crinkle" was a different disease, and that Up-to-Date streak is not a constituent of normal crinkle. As indicated by Murphy and M'Kay (5 and 6), varieties of crinkle exist, one of which the crinkle in Myatt's Ashleaf used by Smith may have been, or it may have contained an additional accidental virus. The latter view is held to be the more likely. As to the nature of this accidental virus, it is at least possible to say that Up-to-Date streak was absent, judging by the (primary) reactions to the complex of four varieties of potato, as determined by Smith. The other reactions given agree with those of crinkle, but, in view of the facts stated above, the strong probability cannot be excluded that an additional unknown virus was present which did not reveal itself on the differential plants employed. It may have been another variety of streak, which has long been known to exist, but the specific reactions of which are not known in detail.

On the assumption that we are dealing with a complex different from crinkle and of unknown composition, the question of the presence of a necrotic Y virus common to Up-to-Date streak loses much of its interest. It is much more important to examine the question whether this streak has any part in the constitution of standard crinkle. It may be said at once that the weight of evidence, which is summarised in the next section, is opposed to this view. The only evidence in favour of it is the apparent similarity of the Y viruses derived from Up-to-Date streak and from a form of crinkle of uncertain composition, respectively, after passage through tobacco. This identification is based on the symptoms produced in tobacco and President potato, and it is

possible that a more extended comparison might reveal differences. On the whole it may reasonably be said that even if the Y viruses in question are identical, this does not constitute proof of the close relationship of crinkle and Up-to-Date streak. To do so it would be necessary to show that these viruses represented an essential element of the two diseases as they occur in the potato. Some proof has been presented that this is not the case, and, until the contrary is demonstrated, the present writer believes that the evidence in favour of the independent natures of streak and crinkle, based on the study of these diseases in a large number of potato varieties, cannot be set aside.

The constitution of crinkle, with special reference to streak.

It has recently been shown by Murphy and M'Kay (5 and 6) that crinkle is a compound of simple mosaic and an undescribed virus naturally present in Irish Chieftain potatoes, to which the provisional name of "A virus" was given. The question whether Up-to-Date streak entered into the composition of crinkle was also considered by these authors, and answered in the negative. Salaman (7) also found no indication of the presence of a streak in this disease. The evidence already presented is summarised here, but the reader is referred elsewhere (6) for a fuller account of the matter.

As shown in the following table, Irish Chieftain is a carrier of Up-to-Date streak, and the addition of this disease to crinkle in Irish Chieftain does not alter the crinkle symptoms (line 1 of Table II). The addition of the Up-to-Date streak to Irish Chieftain carrying the latent virus A produces a crinkle disease (line 2); *but the addition of streak-free Up-to-Date to the same Irish Chieftain produces exactly the same result* (line 3). Both the streak Up-to-Date and the streak-free Up-to-Date are known to carry a simple mosaic,[5] and it is evident that it is the latter disease which produces crinkle-like symptoms in Irish Chieftain by combination with the latent Irish Chieftain virus, irrespective of whether streak is present or not. When streak is present, it is a latent superfluity, not altering the symptoms of crinkle on Irish Chieftain, but revealing itself when this impure crinkle is transferred to President, which is intolerant of streak (line 2—compare line 3).

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[5] The presence and absence of streak, respectively, in these two stocks of Up-to-Date have been repeatedly proved by grafting experiments, extending over four years. The additional presence of a simple mosaic in the streak stock has been noted over and over again by the appearance of mosaic in grafted plants before streak symptoms appear, and by the reappearance of mosaic in plants which recover from streak, as even such highly susceptible varieties as President and Arran Victory sometimes do. The presence of a simple mosaic (and the absence of streak) in the other stock of Up-to-Date has also been proved by repeated grafting on mosaic- and streak-susceptible varieties, including President and Arran Victory.

TABLE II.—Combinations of various diseases in Irish Chieftain.

No.	(*a*) Diseases originally present in— Irish Chieftain.	(*b*) Diseases originally present in— Up-to-Date.	(*c*) Symptoms produced on Irish Chieftain by the combination of diseases listed in Cols. (*a*) and (*b*) opposite.	(*d*) Disease as recovered on President from Irish Chieftain.
1	Crinkle	Streak + a simple mosaic	Crinkle, unchanged	Not attempted
2	Irish Chieftain virus (A)	Streak + a simple mosaic	A form of crinkle	A form of crinkle and streak
3	Irish Chieftain virus (A)	A simple mosaic	A form of crinkle	A form of crinkle alone

The conclusion that streak has no part in the constitution of crinkle is verified by the results of other unpublished experiments. The combination of (streak + simple mosaic) in Up-to-Date with simple mosaic in President and Arran Comrade, respectively, produces in both of the latter varieties symptoms of streak and simple mosaic, persisting for two years, but no crinkle. The combination of the latent Irish Chieftain virus with simple mosaic in President always produces crinkle, as has been shown (6). The Irish Chieftain virus is probably related to the streak group, but so far as its reactions have been determined on differential potato varieties, it is quite distinct from Up-to-Date streak. This is especially clearly seen on streak-free Up-to-Date (which, however, carries a simple mosaic, no virus-free stock being available), to which the Irish Chieftain virus (A) is fatal. As, *ex hypothesi*, virus A should produce crinkle in Up-to-Date carrying mosaic, it is interesting to note that crinkle actually has a fatal effect on this variety also.

The only main-crop[6] variety of potato at present known on which both streak and crinkle produce general necrotic effects is British Queen. In all other known cases the few varieties on which crinkle produces general necrosis carry streak; and those on which crinkle assumes its normal appearance, general necrosis being absent, fall into two groups, one of which carries streak, and the other shows it normally. To virus workers who are familiar with the reactions of potato varieties to these diseases, the independent and distinct nature of Up-to-Date streak and crinkle is scarcely open to question. As shown in the following table, the symptoms of the two diseases are entirely different on most varieties. Attention may also be drawn to the fact, of which an example has already been given, that the presence of crinkle does not prevent the normal symptoms of streak from appearing on varieties intolerant of the latter disease. The same is true of combinations of streak with simple and interveinal mosaic, respectively.

[6] There is a group of early varieties on which both simple and interveinal mosaic, as well as crinkle and streak, produce general necrosis.

TABLE III.—Differential reactions of some potato varieties to crinkle and streak.

Variety and Group.	Symptoms of crinkle.	Symptoms of streak.	Variety and Group.	Symptoms of crinkle.	Symptoms of streak.
Up-to-Date group—	General necrosis, often fatal except in case of Kerr's Pink	Carried without symptoms	Champion group—	Normal crinkle symptoms, without general necrosis	Carried without symptoms. (Some primary streak at times)
Up-to-Date			*Champion*		
Great Scot			*Majestic*		
Kerr's Pink			*Arran Chief*		
President group—	Normal crinkle symptoms, without general necrosis	Severe streaking, usually fatal	*Arran Consul*		
President			*Arran Banner*		
Arran Victory			*Flourball*		
Arran Comrade			*Irish Queen*		

Constitution of virus complexes as determined by synthesis and analysis.

From a number of examples already given it is evident that many dangers are inherent in attempts to determine the constitution of virus complexes by analysis. Attention has already been drawn to this on other grounds (5). *Analysis does not distinguish between a virus present in or with a complex, which is only an accidental or latent superfluity, and a virus which is essential for the production of the symptoms characteristic of the complex.* Determination by synthesis is clearly much less liable to error, and, if an analysis is made, it must be confirmed by synthesis of the given complex, *with the production in the original host, by means of a combination of the smallest possible number of viruses, of a disease recognisably similar to the original complex, and with the same transmissibility and other characteristics.*

The most useful function of analysis is to provide materials with which a synthesis can be accomplished, and the use of indicator plants which appear to interfere with this result is open to objection. Tobacco, no doubt, possesses certain advantages which probably make it indispensable for the study of potato viruses. To rely on it too exclusively, however, to the neglect of other indicator plants, and particularly of the potato, is likely to lead to confusion. A serious objection to tobacco is the unexplained changes which certain, at least, of the potato viruses or complexes appear to undergo when inoculated into it, as a result of which it is apparently impossible to reconstitute the original potato disease with them again. This matter is discussed more fully later, but it may be said here that work based on diseases originating from potato, but radically altered by tobacco, however valuable the contribution to the general

virus problem, is not a study of the original potato diseases, and adds little towards a solution of the practical problems which they present.

It is intended to show in a forthcoming publication how it is possible, by the use of a number of differential potato varieties, to distinguish with precision between various potato virus diseases and complexes, without the complication which may be involved by passage of the viruses through tobacco or other plants which produce similar effects.

The constitution of crinkle as deduced from Salaman's experiments.

Salaman (7) also investigated the same crinkle which we have always used, describing it as "crinkle A." This designation is admittedly a synonym, and should accordingly be dropped. He produced evidence that, following certain methods of inoculation, the crinkle assumed the aspect of a simple or interveinal mosaic, and hovered between two explanations of this phenomenon. It was considered that there was evidence that crinkle is a mixture of viruses, and that there was also evidence of a modification of the virus by dosage (*i.e.*, the varying amounts of inoculum employed in different methods of transmission), and by passage through certain plants. Either of these explanations would account for the experimental results, and it is not likely that both were operative. These results, properly interpreted, are, in fact, in full agreement with the conclusion of Murphy and M'Kay (5 and 6), that crinkle is a complex, and that the constituents are virus A from Irish Chieftain and simple mosaic. In making this interpretation two points must be borne in mind:—

(1) Simple mosaic, while a benign mosaic on most potato varieties, produces lethal or serious necrotic streak-like effects on Epicure and Arran Crest; and

(2) It is doubtful if the fine distinction noted between the symptoms produced on *Datura Stramonium* by crinkle and simple mosaic, respectively, can be used as a certain means of distinguishing the two diseases, unless supported by other evidence. Some other experiments (7, p. 77) support such a view.

The explanation of the experimental results now offered is as follows:—

Datura is entirely immune from the Irish Chieftain virus (A), even when inoculated by means of a graft from crinkle (7, p. 70), but it takes a mosaic disease from the crinkle complex, which is characterised by a mild mottle on all the potato varieties used, except Epicure and Arran Crest, on which streak-like symptoms are produced. This is exactly the effect which simple mosaic produces, and it does not indicate that the disease is still essentially a crinkle, though in a reduced condition (7). It also agrees closely with the effects of interveinal mosaic, but necrotic lesions in President tubers would be expected

in this case. The possibility that interveinal mosaic is a constituent of some "crinkles" is not, however, excluded (6). *Datura,* therefore, separates the Irish Chieftain element from a mosaic element, excluding the former completely.[7] This mosaic is described as a reduced crinkle, "essentially the same as that in President crinkle A itself," mainly on the basis of a slightly different effect on *Datura,* all its other characteristics (as shown above) agreeing with those of simple mosaic (or possibly interveinal mosaic), and not with crinkle. In the light of this contradiction, it may be held as more than probable that the distinction observed between the effects of crinkle and simple mosaic on *Datura* is too slight to differentiate the diseases.

This simple explanation renders unnecessary the view that the crinkle disease, while remaining essentially unchanged, was modified by dosage, or by passage through another plant. It is possible, however, that in the case of the "double grafting" experiments the Irish Chieftain virus might survive long enough in a *Datura* stem to enable it to pass through it to the potato stock below, although being unable to establish itself permanently in *Datura.* What appears to be an analogous case may be quoted. The variety Kerr's Pink, following inoculation with crinkle, develops necrotic primary symptoms on the foliage and tubers, and at this stage a serious mosaic resembling crinkle can be recovered from it. In the following year the Kerr's Pink generally shows a mild mosaic only, and only a mild mosaic can be recovered from it. It is concluded that virus A is responsible for the necrosis, and that it exists for a limited time in Kerr's Pink, but is afterwards entirely thrown off (6). Simple mosaic produces no necrotic symptoms, either primary or secondary, in this variety, and the appearance it presents is quite different.

The possible relationship between crinkle and rugose mosaic.

Valleau and Johnson (13) and Koch (4) have recently shown independently that the disease of potatoes known in America as rugose mosaic is a complex made up of the "healthy-potato" mottle virus and an aphis-transmitted vein-banding virus. Johnson (3), as well as Valleau and Johnson (13), have expressed the opinion that rugose mosaic and crinkle are probably identical, and with this view the writer is on the whole disinclined to agree. In a comparison of American and European potato virus diseases,[8] which was undertaken some years ago, the general conclusion was reached that the two diseases were not

[7] According to K. M. Smith (12), the mosaic element which *Datura* isolates out of the simple mosaic present in crinkle is the X virus. As seen from Salaman's experiments, this virus is highly necrotic to Epicure and Arran Crest, while it may be concluded from a remark of Smith's that Y causes a non-necrotic mosaic in Epicure (12, p. 264). This is in full agreement with the characteristics of X and Y from simple mosaic, as deduced in the present paper, Y being the non-necrotic virus in this case. It is probable, therefore, that the two potato varieties, Epicure and Arran Crest, offer a means of distinguishing the X and Y viruses.

[8] Thanks are due to Dr. E. S. Schultz for kindly supplying the American material.

identical, although somewhat similar. American mild mosaic in Green Mountain was more like crinkle than rugose mosaic. The universal presence, however, of the "healthy-potato" virus in the American material seriously interfered with the comparison on our best differential varieties. So much was this the case that the results were not deemed worthy of publication at the time, but in view of recent events it is proposed to publish an account of the investigation for what it is worth.

The latent viruses present in American "healthy" potatoes express themselves predominantly on many Irish varieties as a fatal streak, which appears to be identical with Up-to-Date streak. In addition, there is commonly present a mosaic which is almost or entirely imperceptible on such a variety as Irish Cobbler, but which is clearly seen on some of our varieties, and which is equivalent to simple mosaic. Simple mosaic, therefore, occurs in America, but it has not apparently been observed or described there on potatoes. The American mild mosaic is obviously not identical with our simple mosaic, for it is a much more severe and striking disease. At the time this work was in progress we tended to equate the "healthy-potato" virus with Up-to-Date streak, neglecting the simple mosaic element, but in view of recent results it is doubtful if this was correct.

Johnson (1) originally isolated on tobacco from supposed healthy potatoes three viruses, described as "mottle," "ring-spot," and "spot-necrosis," but he later showed a tendency to merge mottle with ring-spot, apparently (2), or with spot-necrosis (3). The original conclusion appears to have been the correct one. According to the latest work from his laboratory, Johnson's surmise that spot-necrosis of tobacco is identical with rugose mosaic of potato has been confirmed. It has been shown by Koch (4) that both these diseases are complexes of a mottle virus derived from "healthy" potatoes (which is transmitted by sap, but not by aphis) and a vein-clearing virus (transmitted both by aphis and sap). It will be observed that the "healthy-potato" virus and "mottle" are now specifically identified. It may be surmised that there are affinities between some of the elements of "mottle" and Smith's virus X, and similarly between Koch's aphis-transmitted virus and Y. Both of these viruses would be expected from such an analysis as was carried out by Koch of a potato plant presumed to contain latent simple mosaic. The question remains as to what happened to the streak element in this analysis, and what essential part, if any, it took in the subsequent synthesis of rugose mosaic. If streak is essential to the rugose mosaic complex, then the latter is distinct from crinkle; but if the streak is superfluous or absent, the two complexes may be at least closely related.

It would not be profitable to pursue this matter further at present. It is clear that two viruses, the characteristics of which on tobacco are known, can combine to form rugose mosaic. These viruses originate from potato complexes which are presumed to contain at least simple mosaic and streak, as well

probably as other elements, but it is not known from which of these three possible sources they are derived. Until this information is available it will not be possible to say which potato diseases go to make up rugose mosaic, or whether the latter is identical with crinkle or not.

Nature and nomenclature of potato viruses altered by passage through tobacco.

The question of the nature and nomenclature of potato viruses which have been altered by passage through tobacco, with the result that they appear to produce a different disease on return to potato, is a difficult one. Alterations of this sort have been principally described by K. M. Smith, but in analogous work in America somewhat similar changes have been observed, though these do not appear to be of the same order of magnitude. It is not possible to deal adequately with the American investigations on account of uncertainty as to the exact viruses involved, although it is probable that the latter correspond with European potato viruses for the most part. The general conclusion, however, to be drawn from the work of Johnson (1) is that some intensification occurs of the viruses derived from "healthy" potatoes, including "spot-necrosis" in particular, and also "mottle" and "ring-spot." This was especially observed in the first two or three transfers through tobacco, but not further up to the tenth transfer. There was also evidence that the more virulent ring-spot becomes less malignant after three subsequent transfers through potato. The impression conveyed is that the alteration produced is not of a radical order. Johnson recognised that the result may be a consequence of a change in concentration of the virus; and it is even more likely that it may arise from a change in the relative concentrations of the two or more viruses which are now known to make up spot-necrosis, or rugose mosaic (13 and 4). Valleau and Johnson (13) also record a slight intensification after a two-year sojourn in tobacco of the virus derived from American "healthy" potatoes. From the account given, the virus in question appears to be a complex, including both a ring-spot and a vein-banding element. Further, both the latter workers and Koch (4) were apparently able to synthesise typical rugose mosaic in potato out of simpler elements, which had presumably been passed through tobacco.

In the summary of K. M. Smith's work contained in the following paragraphs the disease produced by needle-inoculation from various potato mosaics is generally identified as (X + Y), while that produced by aphis-inoculation from the same sources is designated Y. This is in harmony with Smith's own interpretation of his work as developed in his latest papers (11 and 12). It should be stated, however, that the possibility of the occurrence of a third factor in some of these diseases is not excluded (12).

Following Smith, we find that the needle-borne viruses (X + Y) derived from passage of a simple potato mosaic through tobacco produced on potato a disease of the same type as the original mosaic, but distinguished as being

intensified, with brilliant yellow mottle and necrotic spots, and as being enormously more infective. The corresponding aphis-borne Y virus produced a similar effect, without the necrotic spotting (8). The characteristic appearance and increased infectivity of the (X + Y) viruses were retained in the second season, which indicates that the disease was permanent, and not subject to further alteration in potato (9). Diseases from the same original source (a mild mosaic in potato) made more virulent by repeated passage through tobacco reproduced the same features as above, but in an intensified form. In this case, in addition to intensified mosaic and necrosis, (X + Y) caused a rapid shrivelling of the leaves from below upwards, usually ending in the death of the plant; and Y caused a symptomatically identical disease, but without the lethal principle which was carried by the needle (9).

In the case of "crinkle from Myatt's Ashleaf," (X + Y) after passage through tobacco produced "crinkle" in (President) potato as in a graft-inoculated plant, but followed by severe "leaf-drop streak," which rapidly destroyed all the leaves but the terminal ones. This disease could be passed on unchanged to other potatoes both by needle and *Myzus persicae* with the greatest ease, in both of which particulars it differed from the original crinkle. The corresponding aphis-borne Y virus from the same crinkle also produced "crinkle and leaf-drop streak" in President (10).

The needle-borne virus from interveinal mosaic after passage through tobacco produced on potato "interveinal crinkle together with the concomitant streak lesions" (10). The reaction of the aphis-borne virus from interveinal mosaic was not given.

Virus X from Up-to-Date streak when passed through tobacco gave rise on potato to dark red local lesions, followed by mosaic mottling, sometimes accompanied by crinkling and distortion. Lesions of the "streak" type were usually absent. The corresponding virus Y produced the same symptoms as in the case of crinkle ("crinkle and leaf-drop streak") (12). The reaction of (X + Y) was not given in this case.

Judging from these descriptions it must be concluded that in no case did any constituent of the original potato virus (or complex), or the sum of its supposed constituents, exactly reproduce the disease on potato in its original form after passage through tobacco. Not only were the symptoms altered in all cases, but the alteration appeared to be permanent. Along with this there usually went increased infectivity both by aphis and needle, and this feature also appeared to be retained into a second season at least. To take an example: If the disease produced in potato by passing "a crinkle from Myatt's Ashleaf" through tobacco is to be considered as "crinkle and leaf-drop streak," it differs both from the original crinkle (and standard crinkle) and from Up-to-Date streak in being readily transmitted in an unchanged condition from potato to potato by needle and by *Myzus persicae*. Are we then to say that crinkle and streak are transmitted by this aphis or not? They are, and they are not. For

there is no conflict of opinion that *Myzus persicae* does not transmit either crinkle or streak as they commonly occur in our potato fields (12).

On the other hand, it appears to be necessary to preserve an open mind as to whether these induced changes have general validity, or, in other words, if, for example, the same changes may be expected in the case of any simple mosaic or any crinkle. On passing standard crinkle through tobacco Salaman (7) secured a mild mosaic on potato, with no intensification on repeated passage (up to three times). Against this, Smith noted that the intensification of simple mosaic did not begin until after the third, fourth, or fifth passage through tobacco (9). On the whole, however, it is probably easier to reconcile the results secured with crinkle by assuming that the crinkle disease used by Smith contained an additional constituent, or was a different complex. There is also some evidence, with which Smith's earlier work (8) is not in entire disagreement, that simple mosaic may not be materially altered in the passage, which would explain why nothing but a mild mosaic was recovered by Salaman on passing crinkle through tobacco. A similar result has been secured in this laboratory. Whatever may be the facts in these cases it is clear that in all Smith's later work, which included a simple mosaic (9), an interveinal mosaic (10), a "crinkle" in Myatt's Ashleaf (10), and a streak in Up-to-Date (12), the diseases recovered on potato from tobacco differed radically from the originals in appearance and transmission.

In so far as these radical changes take place, the identification of the altered potato diseases with the potato viroses from which they were derived leads to much avoidable ambiguity. It is suggested that the best way out of the difficulty is to consider such altered viruses produced in tobacco by inoculation with certain potato mosaic diseases as distinct entities, no longer the same, either individually or in their sum, as those which produced the original diseases in potato. It is likely that there are naturally occurring potato diseases in certain places or countries which correspond to them, and these will obviously need distinct names. On the other hand, it is clear that the majority, at least, of the diseases thus induced in tobacco do not correspond to our naturally occurring diseases either in appearance or properties, nor are they combinations of them, or intermediates between them. It is only adding to the sufficient existing confusion to identify them with the potato diseases from which they originated, or to use names or descriptive terms for them which may be held to imply a relationship with other existing potato diseases, when no such relationship has been shown to exist. There appears to be no theoretical objection to adopting such a view, for so little is known about the constitution and relationships, proximate and remote, of all the viruses of the plant world that it may well explain their diversity.

The writer considers that the discovery of the X and Y viruses (12) is probably of fundamental importance, and represents one step further on the road to an understanding of the constitution of viruses themselves. It is not

considered as conclusively proved, however, that because a potato mosaic expresses itself in tobacco and other plants as an X and a Y disease it exists in similar dissociated elements in potato, although it may do so. The finding of Y alone in potato certainly points in this direction, but the virus X does not seem to have been so found. If the elements are dissociated, it should be possible to reconstitute the disease in potato by a simple mixture of X and Y. On this question there is some conflict of opinion, but in most, at least, of K. M. Smith's analyses a mixture of the resulting X and Y viruses from tobacco did not produce the original disease again in potato. It may be that in the process of passage through tobacco a potato virus undergoes some change which may be in the nature of a dissociation of an irreversible character. It is as though a highly complex chemical substance were to give rise to two derivatives, both with properties something like the original, which it is impossible to combine again. If the experimental results are well founded, that the passage of a potato virus through tobacco may produce another virus with radically and permanently altered characteristics, it would be difficult to parallel the change thus brought about in any known host-parasite relationship, and it would be easier to explain it by looking on the virus as a chemical substance.

Acknowledgments.

The writer has had the advantage of discussing the subject-matter of this paper with his colleagues, Dr. Phyllis E. M. Clinch and Mr. J. B. Loughnane, knowledge of some of whose unpublished results has helped to determine the course of the reasoning and to exclude alternative explanations. The writer, however, is solely responsible for the opinions expressed.

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Summary.

The common occurrence of viroses of the potato in the form of known or suspected complexes, such as simple mosaic, crinkle, and streak, is emphasized, and the practical importance of these diseases or complexes is pointed out.

The view that mosaic, crinkle, and streak are compounds of the same X and Y viruses, and are therefore closely related, has not been established.

Differences are pointed out between the aphis-borne virus designated Y found in or associated with a mild mosaic, and those associated with Up-to-Date streak, and with a form of "crinkle" in Myatt's Ashleaf, respectively.

It is pointed out that streak in Up-to-Date is usually accompanied by a simple mosaic. On analysis, such a complex would be expected to yield X and Y elements, but there is no proof that these derive from the streak virus. The presence of streak, if carried with Y, may account for the difference between the latter and Y from simple mosaic.

Reasons are given for the view that the form of "crinkle" in Myatt's Ash-leaf used by K. M. Smith differed from standard crinkle, and it is suggested that the Y virus from the former is a complex and carries another virus with it.

Proof is presented that Up-to-Date streak is not a constituent of crinkle.

Determination of the constitution of virus complexes by analysis does not distinguish between a virus present with the complex as an accidental or latent superfluity, and the viruses which are essential for the production of the symptoms characteristic of the complex.

Determination by synthesis is much less liable to error, synthesis being defined as the production in the original host by means of a combination of the smallest possible number of viruses of a disease recognisably similar to the original complex, and with the same transmissibility and other characteristics.

It is shown that the mosaic disease secured by Salaman from crinkle corresponds to simple mosaic, and that the results secured are readily explainable on the grounds that crinkle is a complex of this disease and another virosis. It is not necessary to invoke the theory of modification of crinkle by dosage. An explanation of the double-grafting experiment is also offered.

The viruses present in "healthy" American potatoes include those of simple mosaic, which has apparently not been described in potatoes in America, and of streak as in Up-to-Date. American mild mosaic is not identical with simple mosaic, and is more like crinkle. The identity of rugose mosaic and crinkle is uncertain.

The possible alteration of potato viruses on passage through tobacco is discussed. In so far as these changes take place, the resulting diseases do not correspond to local naturally occurring potato diseases, nor to combinations of them, or intermediates between them. It is confusing and unsound to identify them with the potato diseases from which they originated, or to use names or descriptive terms for them which imply a relationship with other existing diseases, where such has not been shown to exist.

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No. 19.

ELECTRICAL PROPERTIES OF OIL-WATER EMULSIONS, WITH SPECIAL REFERENCE TO THE STRUCTURE OF THE PLASMATIC MEMBRANE.—II.

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It has already been shown that water-in-oil emulsions possess a very high electrical resistance, which, however, may be reduced to about 1 per cent. of its original value by the passage through it of an electric current (7 and 9). In order to measure the original resistance it is, thus, necessary to employ very small currents.

The magnitudes of the changes in resistance which occur have been re-investigated by more suitable methods, and further data are now available as to the causes and conditions under which these changes occur.

The biological importance of the results rests in the fact that they show that a continuous film of "oil," which is naturally relatively impermeable to water-soluble solutes, can be broken and made permeable by an "electric stimulus," a phenomenon which is probably similar to the increase in permeability of living cells brought about by electrical stimulation (5, 6, and 8).

Preparation of the emulsions.

Special significance appears to attach to the fact that the composition of the emulsions previously used was 7 vols. oil to 3 vols. water-phase, since these emulsions alone are stable both when the oil-phase is continuous and when the water-phase is continuous. The causes of this stability are uncertain. The experimental evidence on which the statement is made is given in this section.

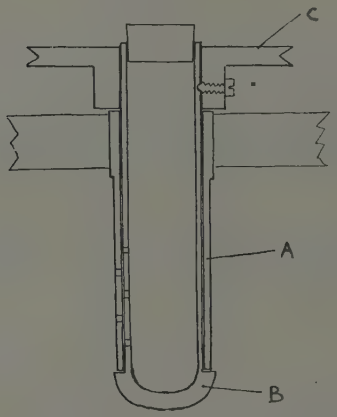
The emulsions were prepared as follows:—The required volumes of oil and water-phase were measured and mixed by shaking by hand. "Pure olive oil" as supplied commercially was used, and the water-phase consisted of N/50 sodium oleate, which was prepared by dissolving 1/50 mol. of pure redistilled oleic acid (B.D.H.) in 1 litre of N/50 NaOH.

The coarse emulsion prepared by hand shaking was "homogenised" by passing it through a simple laboratory homogeniser constructed as follows:—

A stout brass tube (A, fig. 1) is mounted rigidly, and has three holes drilled in its side. A hollow steel tube (B), also with three holes drilled in its side, closed at the bottom, is fitted inside the brass tube. It can be rapidly rotated inside the brass tube by means of the pulley (C) at the top. The three holes in the side of the steel tube do not come opposite to those in the outer brass tube. In the middle region, where the holes are situated, there is a clearance of about 1/1000 inch; at each end of the brass tube the steel tube fits accurately.

The steel tube is filled with coarse emulsion, closed with a cork, and rapidly rotated. The emulsion oozes out, and is carried round in the space between the two tubes, the droplets being broken up by this process; the homogenised emulsion oozes out through the holes in the brass tube.

FIG. 1. FIG. 2.



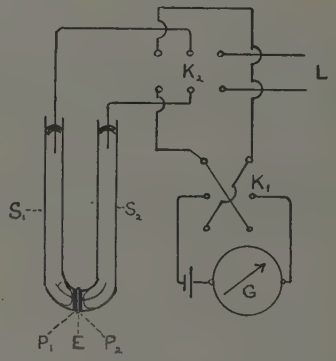


FIG. 1.—Homogeniser seen in median vertical section. A, outer brass tube. B, inner steel tube closed below, which can be rotated in A by means of the pulley, C.

FIG. 2.—Plan of connections used. E, emulsion between two platinum electrodes, P_1 and P_2. S_1 and S_2 glass supports containing mercury. K_1 reversing switch for passing the reading current in either direction through the emulsion, but always in the same direction through the galvanometer, G. K_2 switch for connecting the emulsion with the "stimulating" current, or with the galvanometer. L, leads supplying "stimulating" current.

Experiments A and B.—A series of emulsions were prepared in this manner, containing respectively 10, 20, 30 . . . 80, and 90 per cent. of water-phase. Those containing 20 per cent. or more of water-phase resembled each other in that the oil was present in separate drops distributed through the water-phase. That containing 10 per cent. water had a "curdy" appearance, due to the presence of irregular drops of water-in-oil emulsion distributed through the oil-in-water emulsion.

These emulsions were allowed to stand. Separation of the emulsion into a lower watery layer and an upper layer of emulsion richer in oil took place fairly soon, and after about 12 hours no further separation ("creaming") took place. 100 c.c. samples of emulsion were allowed to cream, and the volume of separated water-phase was noted. The original quantity of water in the emulsion before creaming was known, and the difference therefore gives the quantity of water-phase in the "cream."

It is noteworthy that creaming occurs in all emulsions containing more than 30 per cent. of water-phase, and that the "creams" contain about 20–30 per cent. of water-phase. Table 1, column 1, gives the original composition of the emulsion, column 2 gives the water-content of the separated cream in this particular experiment. Ostwald's explanation of the phenomenon is that non-deformable spheres of uniform volume in the closest-packed condition occupy 74 per cent. of the total space. He regards the dispersed oil-drops as such spheres. Actually, of course, they vary considerably in size, so that in the closest packed state they should occupy more than 74 per cent. of the volume.

A curious phenomenon is observed when these "uncreamed" oil-in-water emulsions are converted into water-in-oil emulsions.

A series of oil-in-water emulsions, containing from 10 to 90 per cent. of water-phase, were prepared and homogenised as described. Each was inverted to the water-in-oil form by the addition of the calculated quantity of calcium chloride (to every 10 c.c. N/50 sodium oleate 0·00007 mol. of $CaCl_2$ was added). After this addition the emulsion was shaken vigorously and then homogenised.

When the original oil-in-water emulsion contained more than 30 per cent. of water-phase, this inversion of the emulsion was always found to be accompanied by a separation of the emulsion into a lower watery layer and an upper emulsion layer, having the oil-phase continuous, and containing roughly 30 per cent. of water. The third column of Table 1 gives the water-contents of the water-in-oil emulsions formed by inversion of the emulsions whose water-content is given in column 1.

TABLE 1.

1.	2.		3.	
Per cent. water in original oil-in-water emulsion.	Per cent. water in "creamed" oil-in-water emulsion.		Per cent. water in "creamed" water-in-oil emulsion formed on inversion.	
Experiments	A	B	A	B
90	46	37	9	8
80	39	34	16	23
70	33	31	25	17
60	30	27	28	6
50	23	26	37	25
40	15	39	35	36
30	26	24	30	30
20	20	20	20	20
10	10	10	10	10

It should be noted that many treatments were employed for the purpose of obtaining water-in-oil emulsions containing more than 30–40 per cent. of water. It was, however, found to be impossible to do this when the emulsion is formed by inversion of an oil-in-water emulsion.

The somewhat erratic figures quoted above from two similar experiments are typical, and they tend to show that the "stable" emulsions contain about 20–40 per cent. of water-phase both when the water-phase is the continuous phase and when it is the disperse phase. If the closest-packing law held, one would expect that the most stable water-in-oil emulsion would contain 74 per cent. of water, not 30 per cent., as is actually found.

Emulsions are not truly reversible if the water content is above about 30 per cent., as the conversion of the continuous-water type to the continuous-oil type is associated under these conditions with the "extrusion" of a certain definite quantity of the water-phase from the emulsion.

It may be added that these remarks apply, of course, to olive oil-water emulsions stabilised with oleates; different quantitative relations are to be expected with other emulsions.

In the work to be described in the following section emulsions were used containing invariably 30 per cent. by volume of water-phase. An oil-in-water emulsion was first prepared as described, using N/50 sodium oleate, and this was inverted by the addition of calcium chloride. A number of emulsions having different sodium : calcium ratios were used.

Methods Employed for Measurements of Resistance.

The resistances were measured by passing a small current through the emulsion and a sensitive galvanometer in series with it.

As a rule, the potential differences applied were between 2 volts and 0·01 volt. These were obtained from a small accumulator and potentiometer.

The galvanometer used was a Leeds-Northrup type R, which gave a deflection (at 1 metre) of 1 mm. with a current of $9{\cdot}7 \times 10^{-9}$ amperes.

The connections used are indicated diagrammatically in fig. 2. The reversing switch (K_1) enables the current to be sent in either direction through the emulsion (E), but always in the same direction through the galvanometer (G). The two-pole switch (K_2) enables the electrode vessel to be connected either with the galvanometer circuit or with the "stimulating circuit," or, alternatively, it can be used to disconnect it from both circuits when the key is left in the vertical position. The details of the stimulating circuit are not shown, as they have been already fully described (5 and 6).

The electrodes are circular pieces of platinum, approximately 5 mm. diameter, with platinum wires fused in at the back (P_1 and P_2). These are fused on to the ends of glass tubes (S_1 and S_2), so that the backs of the electrodes are insulated by the glass. The wire makes connection with mercury, contained

in the tubes as indicated in fig. 1. The exposed front faces of the electrodes (P_1 and P_2) are flat, and the supports are so mounted that these surfaces are parallel and approximately 1 mm. apart. They are blackened in the usual manner. The electrodes are dipped into the emulsion contained in vessels of standard size (1 inch specimen tubes), being held with their faces vertical. This is necessary in order to ensure that the emulsion (sometimes very viscous) displaces the air between the electrodes.

Resistances and certain General Properties of Water-in-Oil Emulsions.

Water-oil emulsions are in general very variable in their properties, although superficially they appear to be quite stable when the relative volumes of the phases are suitably adjusted. The nature of the variations which take place in the electrical resistance can best be illustrated by considering some typical cases.

A series of six emulsions were prepared by mixing 20 c.c. of the stock emulsion (70 volumes olive oil + 30 volumes N/50 sodium oleate) with, respectively, 0·1, 0·2, 0·3, 0·4, 0·5, and 0·6 c.c. of M/10 calcium chloride. These were then homogenised. Their properties are recorded in Table 2.

Emulsions I and II, in which the sodium : calcium is more than 4, are of the oil-in-water type, and were not specially studied in this connection.

TABLE 2.

1. Emulsion number.	2. C.c. M/10 $CaCl_2$ in 20 c.c.	3 Na : Ca.	4. Deflection. Left.	5. Deflection. Right.	6. Sp. R. megohms.	7. Sp. R. 24 hours later.
I	0·1	12 : 1	Oil-in-water.			
II	0·2	6 : 1	Oil-in-water.			
III	0·3	4 : 1	54 46	58 42	1760	610
IV	0·4	3 : 1	42 44	61 44	1830	840
V	0·5	2·4 : 1	49 49	62 67	1540	——
VI	0·6	2 : 1	51 50	43 54	1760	1010

The first three columns give data regarding the composition of the six emulsions. The fourth column gives the galvanometer deflection with the

current sent in one direction through the emulsion, and the fifth the deflection with the current in the opposite direction. Four readings are given for each emulsion; they were taken one after the other, the "zero" reading being taken between each. The variation observed is fairly typical, and it will be noted that, except in the case of emulsion V, the deflection with the current from left to right is of about the same magnitude as when it flows from right to left.

The four emulsions have approximately the same resistance, and the resistance of the fresh emulsion is also approximately the same as that of the oil which forms the continuous phase. These resistances are noted in the sixth column. 24 hours later the resistances were again measured, and the values obtained then are given in the last column.

It will be noted that all the resistances are lower, but that no special regularity is shown as to the extent to which the resistances have fallen, except that the greatest falls have taken place in the emulsions having the largest Na : Ca ratios. The causes of this change in resistance are not known.

General Effects of Larger Electric Currents on Emulsions.

Emulsion VI was first used. The resistance was first measured, and then 100 volts alternating current was applied for 1 second to the layer of emulsion 1 mm. thick. The alternating P.D. was obtained by the use of a potentiometer supplied from the mains, the frequency of which was about 50 cycles per second. The effect of this "stimulus" was negligible. The decrease in resistance which was observed is of the same order of magnitude as the spontaneous fluctuations in resistance which are continually taking place.

The actual resistance-changes are given in Table 3, column 1.

Table 3.

	1. Emulsion VI. 100 v. 1 sec.	2. Emulsion VI. 195 v. 1 sec.	3. Emulsion III. 100 v. 1 sec.
Resistance before stimulus ...	1010	990	610
2 mins. later	960	101	—
3 mins. later	—	68	—
5 mins. later	—	62	—
8 mins. later	—	550	—
13 mins. later	—	960	15

A second sample of emulsion VI was then stimulated with 195 volts A.C. for 1 second as before, and the results are given in the second column of Table 3.

It will be noted that a very rapid decrease in resistance takes place, which is followed by a strikingly rapid recovery to a value close to the original high resistance. The lowest value attained was approximately 7 per cent. of the original resistance.

In the previous communication (9) the cause of this decrease of resistance was found to be the partial inversion of the water-in-oil emulsion to the oil-in-water type, which resulted in large drops of the latter type bridging the space between the electrodes. The emulsions used previously were not homogenised, and their irregular structure was the probable cause of their erratic behaviour. They always contained multiple drops (which have been already noticed by Seifriz (23)), *i.e.*, droplets of oil inside the water-drops which were distributed in the continuous oil-phase. These water-drops were sometimes fairly large, and were almost always irreversibly increased in volume after a stimulus by coalescence with smaller water-drops surrounding them. These unhomogenised water-in-oil emulsions did not, therefore, recover from the effects of the stimulus.

The characteristic feature of the homogenised emulsions dealt with here is that recovery nearly always took place from moderate stimuli.

The course of events was watched under the microscope by placing the emulsion on a slide, between two electrodes fixed 1 mm. apart. The homogenised emulsion VI did not contain "multiple drops," though it may be noted that the size of the drops varied considerably. At the moment of application of the stimulating current (195 volts, 1 second) large numbers of the originally spherical drops became ellipsoid, and arranged themselves end to end in rows roughly parallel with the direction of the electric field. Coalescence of the individual drops in a filament took place in a few places, so that larger roughly spherical drops were formed, but the majority of the drops did not coalesce, and, during the period after the stimulus, the filaments gradually broke up into their separate drops. The drops maintained their ellipsoid shape for a considerable time after the stimulus, a fact which is no doubt related to the presence of a solid soap film surrounding them.

Evidently the formation of narrow filaments of water-phase is responsible for the decrease in resistance, and the recovery to the original high value would seem to be due to the break up of the filaments of drops into separate drops. A very large stimulus (high voltage or long duration) brings about much coalescence of the droplets of the filaments, and the large resulting drops do not break up again, so the effects of a stimulus which is too large are in general irreversible; no recovery takes place.

Emulsion III was also stimulated, and the results are given in the last column of Table 3. 100 volts for 1 second was used, and it will be noted that an enormous fall (97·5 per cent.) in resistance took place. Emulsion III is thus very much more sensitive than emulsion VI, and, as has been pointed out already (9), this is due to the higher sodium : calcium ratio.

Readings of its resistance during the first 15 minutes after the stimulus were impossible, because the potential difference between the two electrodes was great enough to send the spot of light far off the scale. The actual magnitude of this potential difference may be of little significance. Its cause is at present uncertain. The phenomenon is always noted when a very large response is obtained. Moderately large responses (*e.g.*, emulsion VI, 195 volts, 1 second) are rarely accompanied by the production of this potential difference between the electrodes.

Effects of Liminal Stimuli, and Behaviour of very Sensitive Emulsions.

The emulsions considered in this section were richer in water than those previously dealt with; they contained somewhat more than the maximum water content compatible with stability, namely, 45 per cent.

Emulsion VII was made up of 40 c.c. stock; 8 c.c. M/100 $CaCl_2$; and 5 c.c. water. The sodium : calcium ratio was thus 3 : 1, and the oil : water ratio 30 : 25.

Emulsion VIII was made up of 40 c.c. stock; 10 c.c. M/100 $CaCl_2$; and 3 c.c. water. The sodium : calcium ratio was 2·4 : 1, and the oil : water ratio again 30 : 25.

Emulsion VII was examined when freshly prepared, 10 minutes after homogenisation, and at various periods subsequent to this; namely, when 3–5 hours old; 24 hours old; and 48 hours old. A very marked increase in sensitivity took place during the first five hours after the preparation of the emulsion. (Cf. 9.)

This will be made clear by reference to the following data obtained from experiments on emulsion VII. The cell-constant was 4·4, hence the specific resistances are 4·4 times the values given here.

Emulsion VII was homogenised at 12.50 p.m. Its resistance was 450 megohms. When stimulated with 50 volts A.C. for 1 sec. at 1.10 p.m. its resistance fell in 2 minutes to 255 megohms (*i.e.*, 44 per cent. response). The resistance recovered after 1 hour 40 minutes to the original magnitude, actually to about 500 megohms.

Another sample of same emulsion 2 hours 10 minutes old had a resistance at 3 p.m. of 666 megohms. A stimulus of 80 volts A.C. for 1 sec. brought the resistance to 31 megohms (*i.e.*, response of 95·4 per cent.).

At 4.10 p.m. the resistance was measured, using a P.D. of 0·25 volt to send measuring current through the emulsion; a deflection of 13 mm. was obtained, corresponding to a resistance of 192 megohms. The resistance was again measured, using a P.D. of 1·0 volt; a deflection of 116 mm. was obtained, corresponding to a resistance of 86 megohms. Again using 1·0 volt, the apparent resistance was found to be 44·5 megohms. A measuring voltage of 0·25 was then used, and the resistance found to be 78 megohms.

Evidently the measuring voltage of 1·0 volt has been sufficient in this case to cause a decrease in resistance of the emulsion. The resistance of the *fresh* emulsion (obtained by applying Ohm's law) was the same whether the measuring voltage was 0·25, 1·0, or 2·0 volts. This is no longer the case with the 3 hour-old emulsion, as these voltages evoke considerable responses.

Another sample of the same emulsion had a resistance of 800 megohms at 5 p.m.

It was stimulated with 5 volts A.C. for 1 sec. The resistance fell to 165 megohms (response 80 per cent.).

Thus an emulsion when four hours old gave a bigger response to a stimulus of 5 volts than was given by the same emulsion freshly made up to a stimulus of 50 volts.

Two days later the 45–50 hour old emulsion was investigated. The sensitivity appeared to have remained fairly constant during the period of six hours during which the experiments were continued. The magnitudes of the responses obtained, using various stimulating voltages, are recorded below.

TABLE 4.

Responses of emulsions 50 hours old after 1 second's stimulation at different voltages.

Emulsion VII.						Emulsion VIII.	
Voltage.	Response.	Voltage.	Response.	Voltage.	Response.	Voltage.	Response.
1·0	− 5	1·5	− 15	2·5	− 10	10·0	+ 1
1·0	− 3	1·5	− 14	2·5	+ 1	20·0	0
1·0	+ 3	1·5	+ 6	2·5	+ 3	35·0	0
1·0	+ 2·6	1·5	− 6	3·0	− 16	40·0	+ 2
1·5	0	2·0	− 15·5	3·5	− 45	45·0	− 90
1·5	− 2·6	2·0	− 7	4·0	− 86	60·0	− 97
1·5	− 2·5	2·0	− 6	5·0	− 95	—	—

These results are shown graphically in fig. 3. Responses are plotted on the vertical axis and the applied voltages on the horizontal axis.

It will be noted that, although a considerable amount of irregularity is present, the results fall roughly on an S-shaped curve.

As has been pointed out before (8), the simplest explanation of this is that it is a statistical effect, due to the presence of droplets of water-phase of different compositions dispersed through the oil-phase.

One presumes that the decreases of resistance to the extent of 95–99 per cent. are brought about by the deformation of almost all the droplets, which are

thus caused to form chains like strings of elongated beads between the electrodes. Smaller responses correspondingly are brought about by the deformation of a smaller number of droplets.

Droplets in an emulsion containing a high Na : Ca ratio and still under the inversion point are apparently more readily deformable by a given force than those of lower Na : Ca ratio; comparison of the results obtained with emulsions VII and VIII exemplify this point (Table 4, fig. 3). Though the precise

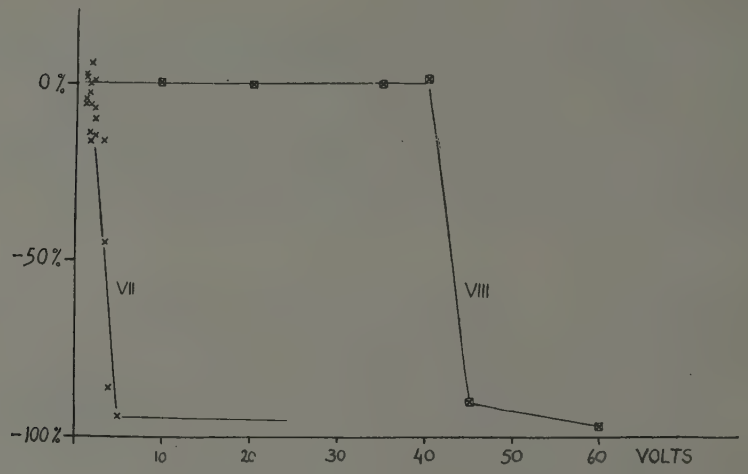


Fig. 3.—Graphic representation of Table IV. The responses of the two emulsions, VII and VIII, are plotted on the vertical and the applied voltages on the horizontal axis. The emulsions were of the same age. The sodium : calcium ratio of VII was 3 : 1 and of VIII 2, 4 : 1.

reason for this is not clear, it seems not unlikely that it is connected with the fact that emulsions of sufficiently high Na : Ca ratio become inverted to the oil-in-water type.

The S-shaped response-voltage relation can be explained as the counterpart of a normal frequency distribution curve of Na : Ca ratios of the water-droplets of the emulsion.

The Effects of Temperature on the Sensitivity of Emulsions.

Experiments on the effects of temperature have been merely of a preliminary character.

For example : Emulsion IX was made by homogenising 20 c.c. of stock with the addition of 6 c.c. of M/100 $CaCl_2$. The resulting Na : Ca ratio was 2 : 1.

A stimulus of 45 volts A.C. for 1 sec. at 21° C. brought a drop in resistance of 34 per cent. After recovery to the original resistance, the temperature of the emulsion was raised to 55° C. In 15 minutes it was again stimulated for 1 second with 45 volts A.C. The resistance fell to 3 per cent. of the value before the stimulus.

Emulsion X was investigated similarly. In this case the Na : Ca ratio was 2·67 : 1. The results obtained are tabulated below.

TABLE 5.

Emulsion X at 21° C.		Emulsion X at 63° C.	
Voltage.	Response.	Voltage.	Response.
20	0	5	0
35	0	10	– 43 %
40	0	10	– 28 %
45	– 90 %	20	– 99 %
		20	– 97 %

The temperature is seen to have a very marked effect on the sensitivity of the emulsion to electric stimulation. The exact nature and causes of the effects were not specially investigated.

It was provisionally imagined that decrease in viscosity of the two phases of the emulsion would greatly increase the deformability of the droplets, and thus the sensitivity of the emulsion. Moreover, surface tension effects and changes of Na : Ca ratio at the interface of a nature unknown at present are also likely to take place.

Summary of Experimental Results.

Oil-water emulsions containing much more than 30 per cent. of the water-phase are unstable and are not truly reversible.

The application of quite small voltage-gradients across water-in-oil emulsions brings about the formation of columns of the water-phase through the emulsion by the elongation of the droplets along the electric field.

The formation of these columns, even without inversion, results in a very large increase of the electrical conductivity of the layer of emulsion, and, of course, immediately produces the effect that the permeability of the emulsion layer between the electrodes for water-soluble substances has greatly increased.

Automatic recovery of the original resistance was observed in homogenised emulsions.

The passage of a current often leaves water-in-oil emulsions polarized, and the potential differences produced may be retained for a considerable time.

It has been shown, that, with suitably adjusted Na : Ca ratio of the emulsion, very small voltages bring about the puncturing of the oil-layer by the deformed water-phase. Thus a voltage-gradient of 1·5 volts per millimetre produced a fall in resistance of about 15 per cent., whilst 4 volts per 1 mm. brought about a fall in resistance of about 86 per cent., at a temperature of 21° C.

Within certain limits, it has been found that decreasing the Na : Ca ratio of water-in-oil emulsions decreases their sensitivity.

Further, it has been found that a rise in temperature of the emulsion increases its sensitivity to a very considerable extent: Thus an emulsion which gave a 50 per cent. response with 43 volts at 21° C., gave a 50 per cent. response with 10 volts at 63° C.

Finally, a marked change in sensitivity of emulsions on ageing was observed.

General Discussion.

The foregoing contains further evidence that oil-water emulsions behave electrically in a manner similar to the plasmatic membrane. The points of similarity may now be summarized as follows:—

1. The passage of a small current may lead in each to a small rise or fall of resistance.

2. The passage of a large current in both is followed by a rapid fall in resistance. This is succeeded by a slow rise until more or less complete recovery is attained. The magnitude of the fall of resistance varies in each with the voltage, the duration and the frequency of the applied current, from 0 to 99 per cent.

3. In each the response-voltage curve is S-shaped.

4. The magnitude of the change of resistance due to the passage of the current also varies in each with the temperature. The same current produces a larger resistance-change at a higher temperature.

5. Finally, the passage of a current may leave both the emulsion and the plasmatic membrane polarized, and in each case this polarization is maintained for a considerable time.

It is now of interest to enquire how far a plasmatic membrane, having the structure and properties of an oil-water-emulsion-film, might be supposed to account for the more striking observations on cell-permeability and on other properties of cell-surfaces.

The salient features of cell-permeability hitherto observed may be summarized as follows:—

1. Very great resistance is opposed to the entry of water by the plasmatic membrane, so that under a pressure of one atmosphere the actual rate of streaming is as little as 33μ per hour (Huber and Höfler, 12).

2. Still higher resistance is opposed by it to the passage of water-soluble non-electrolytes. In these cases the resistance may be 120–10,000 times as great as that opposed to water (Huber and Höfler, 12), and the magnitude of this resistance is a function of the molecular size of the substance considered. (Rühland and Hoffman, 21; and Schönfelder, 22.)

3. The resistance experienced by lipoid-soluble substances is low compared with that for water-soluble substances, and in this case the molecular size is not an important factor. (Collander and Bärlund, 3; Bärlund, 1; Schönfelder, 22.)

4. A very high resistance, higher than any of the above mentioned, is opposed to the entry of electrolytes.

We may suppose the surface film of a cell to be normally composed of a water-in-oil emulsion, for we know that there are physical forces tending to bring the lipoid particles to the surface of the cell and the presence of emulsifying agents in the protoplasm will cause the emulsification of the film so formed. Microscopic observation proves that this film is of extreme tenuity—in most cases beyond microscopic vision. Fricke and Morse (10) and M'Clendon (17), from their data of the relationship between impedance and frequency of current, have calculated the value of the capacity in series with the resistance of the cell-interior in the case of blood corpuscles. If it be assumed that the dielectric is fat or oil, the thickness of the dielectric can be calculated (the dielectric constant of fat being known). The thickness of the film is thus calculated to be somewhere between 0·4 and 4·0 $\times$ 10^{-7} cm., about 3–30 carbon atoms in thickness. Not only does this work (cf. Philipson, 19) dispose of the objection that the electrical conductivity is more influenced by changes in concentration of the cell-sap than by changes in permeability (see for example Stiles 24, p. 180), but it yields evidence in favour of the view that the plasma-membrane consists of a lipoid layer of extreme tenuity—a few molecules thick.

From these dimensions we may assume that the droplets of the disperse phase of the emulsion—normally water—will be of molecular dimensions. It is of interest that Huber and Höfler (12), from measurements of the rate of flow of water across the plasma-membrane during plasmolysis, have come to a similar conclusion.

Thus the water-in-oil emulsion may be pictured as a film of oil penetrated by pores of molecular dimensions filled with the water-phase. Such a structure will evidently act as a molecular sieve, and will tend to oppose a resistance to the passage of water-soluble substances proportionally to their molecular size. Hence it is observed that water passes through the film with comparative ease, and the resistance experienced by water-soluble substances is a function of their molecular size.

The relatively small resistance experienced by lipoid-soluble substances depends on the fact that the continuous oil-phase presents a comparatively unobstructed path to applied lipoid-soluble substances. In this connection it must be remembered, as Irwin (14) has pointed out, that the passage of a substance from the external watery solution through a lipoid layer into the watery plasma within does not depend solely on its lipoid solubility. It depends rather on the values of the partition-coefficients of the external fluid : the lipoid phase, and that between the lipoid phase : the plasma within. If the latter partition-coefficient (lipoid : plasma) is very high, the substance will not enter the plasma appreciably, however lipoid-soluble it may be : it will simply remain concentrated in the lipoid layer.

Stated in another way; so long as a substance is soluble at all in the membrane, its precise lipoid solubility is immaterial, and its final distribution inside and outside the cell will depend solely on the partition-coefficient of the cell-contents : external medium.

No doubt certain substances, whose lipoid : water partition-coefficients are suitable, are able to utilize both paths through the film, viz., the water-phase and the lipoid-phase. The resistance they experience will then be determined in part by their lipoid : water partition-coefficient and in part by their molecular size.

The very high resistance experienced by most electrolytes in passing through the emulsion film is readily explained by the negative adsorption of these substances on the surface of the pores. Tinker (25) has shown that the semipermeability of precipitation membranes is quite explicable as due to the preferential adsorption of water on the pore-wall, hence filling the pores with water and rejecting the solutes. It may be also pointed out that the ions are surrounded by a shell of water-molecules, so that the opposition they experience considerably exceeds that which would be anticipated from their actual size.

It may be noted here that the water-soluble non-electrolytes which pass through the water-filled pores of the emulsion-membrane are to a greater or less extent surface-active, and hence are able to enter the pores. The few exceptions which have been observed to this rule are doubtless explicable on the fact that their surface-activity has been measured on the water-air interface and not on a water-lipoid one.

Clowes was the first (2) to point out that the facts of antagonism and the behaviour of narcotics could readily be brought into line with the emulsion-theory of the plasmatic membrane. Seifriz (23) showed, however, that some

emulsions are not reversible, and suggested that there is no reason to believe that the emulsion formed at the surface of protoplasts would be of the reversible type.

The work recorded in this paper demonstrates that the raising of the Na/Ca ratio of water-in-oil emulsions decreases the stability of the continuous oil-phase, even without reversal, and so may be expected to increase its permeability. Hence antagonistic effects might be expected in irreversible emulsions.

In conclusion it may be said that the emulsion-theory, which appears to be supported by (1) the observed electrical properties and behaviour of the plasmatic membrane, (2) the broad facts of cell-permeability, and (3) the observations on antagonism, forms a useful working hypothesis for future investigation.

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No. 20.

THE COMPOUND NATURE OF CRINKLE, AND ITS PRODUCTION BY MEANS OF A MIXTURE OF VIRUSES.

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(Plates 10–12.)

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The disease of potatoes known as crinkle was first described by one of the present authors in 1920 and 1921 (5 and 6). Previous to that time it had been believed that the various forms of mosaic observable in the field represented stages in the development of the same disease (14 and 15), and it was the current opinion that a potato plant following infection with mosaic went, during the course of an indefinite number of seasons, through a progressive cycle of developments, of which the first stage would be what is now known as mild or simple mosaic and the last "curly dwarf."

It followed as a natural corollary from the recognition of crinkle as an independent disease that the various manifestations of mosaic in potatoes probably represented separate fixed diseases, and the work of Quanjer (12 and 13) in Europe, and that of Schultz and Folsom (16) in America, in separating and describing the principal forms of mosaic, has proved the basis on which all subsequent work has rested. As time goes on the mosaic diseases are being still further subdivided, or the list extended by the description of new forms, but we are not at present concerned with the later work.

It is shown below that one of the original diseases, namely, crinkle, is not a single disease but a compound of simple mosaic and another apparently undescribed virus disease, which for the sake of convenience is provisionally termed "disease A" below. The corresponding virus is also designated "virus A," until its constitution and relationships have been more exactly determined.

As has already been shown in a preliminary report (11), the evidence in support of this view may be summarised as follows:—

(1) When simple mosaic was introduced into plants of the variety Irish Chieftain naturally infected with disease A, symptoms indistinguishable from crinkle were produced, and these symptoms persisted unchanged for at least two to three seasons. The experiment was repeated three times in different years with the same result. Simple mosaic of Irish and Dutch origins had the same effect.

(2) The crinkle-like disease produced as above in Irish Chieftain when transferred to healthy President produced typical crinkle symptoms in the majority of cases, and faint mosaic in others. The crinkle symptoms persisted unchanged in President for at least three seasons.

(3) When virus A was inoculated into healthy President, and following this simple mosaic was introduced into the same plant, typical crinkle followed in the majority of cases and persisted for two seasons. The same result was obtained from two experiments.

(4) The crinkle-like diseases produced in President according to the methods described in (2) and (3) above both produced the typical and fatal effects of crinkle when inoculated into Up-to-Date.

The investigation was supported by means of a grant from the Empire Marketing Board, which is gratefully acknowledged.

Production of diseases resembling crinkle by addition of simple and interveinal mosaics, and (impure) streak, respectively, to Irish Chieftain carrying virus A.

The present problem arose in the first place from the examination of an apparently healthy plant of Irish Chieftain selected by one of us in 1924 on the farm of Mr. D. M'Kelvie, Isle of Arran, Scotland. Stocks of this variety generally show pronounced symptoms of mosaic more or less throughout, and the selected plant was more nearly healthy in appearance than any seen up to that time. It and its vegetative descendants have been grown since under the usual insect-proof conditions (as well as in the open), and they have retained a comparatively healthy appearance. While in the early stages of growth the presence of mosaic is either faintly visible or suggested, the plants later become practically or entirely normal in appearance and make an excellent growth. In the course of routine work on the production of virus-free stocks of potato varieties,[1] this Irish Chieftain has been repeatedly grafted on the two usual

[1] All potatoes designated healthy in the ensuing experiments have been proved to be free from any known virus, including the so-called "healthy-potato" virus. The absence of the latter is of particular importance in this case, since it has been shown that the "healthy-potato" virus is a constituent of rugose mosaic, a complex which has an apparent similarity to crinkle. The matter is more fully discussed elsewhere (7).

test varieties (9, 10), President and Arran Victory, in the years 1925, 1927, 1929, and 1930. The result of the first test (carried out only on President) was considered negative, leading to the presumption that the Irish Chieftain was virus-free, although it was decided to repeat the experiment. It may be said at once that all subsequent repetitions have shown that a mosaic disease is present in this Irish Chieftain, which is faintly visible not only on the Irish Chieftain itself at times, but which also reveals itself as a form of very faint, but usually recognisable, mosaic on both President and Arran Victory (Figs. 1 and 2).

In the meantime, while re-testing was in progress, the step was taken of inoculating the supposed healthy Irish Chieftain with the purest available forms of virus disease, in order to determine the symptoms of these diseases on the variety. The results are summarised in the following Table (I):—

TABLE I.—Effect of introduction of various virus diseases into Irish Chieftain which was afterwards shown to carry virus A.

Year.	Disease inoculated into "healthy" Irish Chieftain.	Appearance in Irish Chieftain.		Recovery on President.
		Current year.	Subsequent year(s).	
1927	Simple mosaic.	Crinkle-like.	Crinkle-like.	Crinkle in two plants and simple mosaic in one.
1927	Interveinal mosaic.	Crinkle to interveinal mosaic.	Crinkle to interveinal mosaic.	Streak-like: fatal.
1927	Streak (2nd stock).	Streak at first, later crinkle-like.	—	No recovery.
1927	Streak (3rd stock).	Streak at first, later crinkle-like.	Crinkle-like.	No recovery.

Crinkle was already present in Irish Chieftain, so no inoculation was made with it at this time. The result of the other inoculations was very surprising, for a disease with a greater or less resemblance to crinkle was produced in each case. The resemblance varied somewhat at different stages of growth, particularly in the case of interveinal mosaic, the identity of which was not completely lost, especially as the plants grew older. But the symptoms produced by simple mosaic on Irish Chieftain were quite atypical of that disease throughout, and were indistinguishable from crinkle. Somewhat the same thing was true of the two streak inoculations, but these form a special case, and are more fully discussed below.

Examination of crinkle-like disease produced by addition of simple mosaic to Irish Chieftain carrying virus A.

Attention was focussed on the simple mosaic problem. The first explanation to suggest itself was that this disease had been intensified by passage through Irish Chieftain. The "intensified" mosaic reappeared in the same crinkle-like form in Irish Chieftain in the following year (and again in all later years), showing that the change was permanent. The conclusion might, therefore, have been reached that this represented the appearance of simple mosaic in this variety. The precaution has, however, always been taken of "recovering" a disease which has been inoculated for the first time into supposed healthy plants of a new variety, and unless it reappears unchanged on a well-known variety the experiment is considered vitiated. When recovery was effected in this case on three plants of healthy President, a disease similar to crinkle appeared in two cases and a simple mosaic in the other. (Compare Figs. 5 and 6.)

The matter had now taken a new turn, for it appeared as though the simple mosaic had picked up an element in the Irish Chieftain, the addition of which produced crinkle, synthesising that disease; and, furthermore, that the compound passed over to two of the President plants, but only one of its constituents to the other.

The experiment of 1927, in which a crinkle-like disease was produced in healthy-looking Irish Chieftain (which, however, was afterwards shown to carry virus A) by inoculating the latter with simple mosaic, was repeated in 1928 and 1929, and with the same result in each case. The details of all the experiments are summarised in the following table, and some of the results are shown in Figs. 3, 4, 5, and 6:—

TABLE II.—Results of infecting Irish Chieftain carrying virus A with simple mosaic, and recovery of resulting disease on President.

1	2	3	4	5			6		7	8
Year.	No. of Irish Chieftain inoculated.	Source of simple mosaic used to inoculate Irish Chieftain.	Method of inoculation.	Infection produced on Irish Chieftain resembled—			Recovery from Irish Chieftain		Infection produced on President resembled.	Remarks.
				1st year.	2nd year.	3rd year.	By	On		
1927	7.136 [1]	A. V. 6.192 [2]	Top-graft	Crinkle	Crinkle	Crinkle	Core-grafts	President	Two plants crinkle, one mosaic [5]	—
1928	7.96	A. V. 7.131 Pres. 7.140	Core-graft	—	—	—	—	—	—	Inoculation failed
1929	9.158A	A. V. 8.123 [3]	Core graft	Crinkle to mosaic	Crinkle	Crinkle	Top-grafts (1929)	President	Two plants, both faint mosaic [6]	Recovery attempted soon after infection of I. C.
							Core-grafts (1929–'30)	do.	One plant crinkle, one "sub-crinkle" [7]	Recovery made following winter
							Top-graft (1930)	do.	Crinkle [8]	Recovery made following summer by top-graft
1929	9.232	Pres. 8.450	Core- and top-graft	Crinkle	Crinkle	—	Core-graft	do. [4]	Three plants crinkle [5]	—

[1] *Col. 2.*—A sister Irish Chieftain plant was grafted in the same year on Arran Victory, but no crinkle resulted either in that year or the following one, showing that virus A without simple mosaic cannot produce crinkle. A similar result was secured when the same stock of Irish Chieftain was grafted on healthy President in 1929.

[2] *Col. 3.*—The first four sources of simple mosaic were of Irish origin, and constitute our standard stock of this disease. The last one was of Dutch origin, kindly supplied by Prof. Quanjer some years ago. It has always been found identical with our own.

[3] *Col. 3.*—This simple mosaic was inoculated into healthy President direct at the same time, on which it produced simple mosaic, persisting for two years. When the same original simple mosaic was passed through Irish Chieftain to President, crinkle was produced (in part) in the latter (Col. 7).

[4] *Col. 6.*—This stock of healthy President was grafted at the same time on Irish Chieftain carrying virus A, but no change resulted in the latter variety, either in that year or the following one, showing that crinkle is not produced by grafting healthy President on Irish Chieftain.

[5] *Col. 7.*—Not grown in the following year.

[6] *Col. 7.*—The faint mosaic persisted unchanged for 3 years.

[7] *Col. 7.*—The crinkle persisted unchanged for 2 years. The "sub-crinkle," which also persisted for 2 years, was intermediate between crinkle and simple mosaic, and is more fully discussed below.

[8] *Col. 7.*—The crinkle persisted unchanged for 2 years.

As will be seen from Table II, a disease closely resembling crinkle was conveyed to the stock of Irish Chieftain used in three out of four experiments, infection failing apparently in one. It will also be observed that the simple mosaic used included both Irish and Dutch material, in two varieties (col. 3); that the same result was eventually obtained by top- and core-grafting (col. 4); and that the crinkle-like symptoms in Irish Chieftain persisted as long as the plants were grown, that is, for 2 to 3 years (col. 5).

It will also be seen that on the attempted recovery of simple mosaic, as such, in healthy President, only crinkle-like symptoms appeared in the majority of cases (col. 7). This proved to be irrespective of the method of inoculation. It was at first thought, as a consequence of the results obtained in this connection in the first experiment of 1929, in which recovery by top-graft, *carried out soon after the original inoculation of Irish Chieftain,* produced a faint mosaic, while recovery by core-graft (9, 10) in the *following winter* produced crinkle, that the method of infection might be important. Accordingly, still another recovery by top-graft was made in the following year, with the result that crinkle followed. The true explanation, it may be concluded, is that the longer the interval which elapses after the synthesis is made, the more certain are all organs to be permeated by both viruses. It may be that one of the viruses is distributed more rapidly through the plant than the other, for there is a noticeable tendency for one to be lost in recovery even though the effects of both subsequently become visible in the Irish Chieftain. It is also possible that one of the viruses possesses a greater facility for passing from a scion into the healthy stock-plant.

A number of control experiments were conducted at the same time, as noticed in the foot-notes to Table II. It was established in these that neither simple mosaic nor virus A, when used separately, could produce crinkle on President or Arran Victory, and that healthy President did not cause any change in Irish Chieftain carrying virus A. In this connection it may be remarked that Irish Chieftain infected with this virus is an extraordinarily sensitive test plant for simple and interveinal mosaics, and has been used by us for this purpose since 1923 (8). Conversely, the presence of virus A may be shown in the same way.

In one case a peculiar disease was recovered from the artificially produced crinkle in Irish Chieftain, which was intermediate between simple mosaic and crinkle, and is distinguished as "sub-crinkle" in Table II (col. 7). This first appeared in the originally inoculated President plant as typical crinkle in the first part of the season, but later in the same season came to resemble more nearly simple mosaic. The symptoms moderated to a much greater extent than ever takes place in the case of crinkle. In the following year, when the resulting tubers were grown in the field, the plants they produced went through exactly the same cycle of development, showing crinkle in June, but recovering to such an extent by the end of August that they then resembled a simple mosaic more than crinkle, and were still in almost full vigour. At the latter

date all the normal and "synthetic" crinkle plants were practically dead. A similar, and probably closely related or identical, disease has been met with now and again in other experiments.

It would not be wise to generalise on the strength of these somewhat isolated occurrences, but there may be indications here that one or more of the viruses which go to make up crinkle is capable of further analysis, and that the dropping of a constituent may give rise to such an intermediate disease as has been described. The conclusion of K. M. Smith (18) that simple mosaic is capable of being resolved into two constituents may have a bearing on this question.

Separation of the constituents of naturally occurring crinkle in graft transmission, and natural recovery from the disease.

The separation of the artificially produced crinkle into its constituents on graft-transmission in certain cases is not without a parallel in the case of crinkle as it occurs in nature. When the disease is transmitted by means of grafts it behaves as a unit in the great majority of cases, but a considerable number of instances have now been recorded in the course of our investigations in which a constituent of the complex must have been dropped. These cases help to throw some further light on the constitution of crinkle, and they also present points of considerable general interest.

When crinkle is transmitted by grafts from varieties which show this disease in its normal form, such as President and Irish Chieftain, the usual rule is that the combination appears rapidly and in full on the inoculated plants. When the grafts are derived from varieties on which crinke produces a serious necrotic disease, such as British Queen and Great Scot, the infection fails in a good many cases, but any disease which is transmitted is usually crinkle, although the symptoms develop slowly. There is also another group of varieties, of which Kerr's Pink is one example, and Epicure probably another, which appears to take crinkle on artificial inoculation, but afterwards generally fails to yield the full complex.

Two experiments were carried out with Kerr's Pink extending from 1926 to 1929 and 1929 to 1931, respectively, with similar results in both cases. Following inoculation with crinkle the Kerr's Pink developed necrotic spotting and accompanying contortion of the foliage, and serious internal necrosis of the young shoots. This is the usual result of crinkle on this variety, and no other virus disease with which we have worked produces a similar effect. There was also severe tuber necrosis in one of the experiments at least. In both cases the first attempts to recover crinkle from the recently inoculated plants yielded on healthy President a rather severe mosaic disease, which was described as a moderate crinkle. In the meantime, the second generation of infected Kerr's Pink plants had appeared, and the majority showed no necrotic symptoms, even when the plants were derived from necrotic tubers, while one showed a trace

of necrosis in the foliage and in one tuber. When an attempt was made to recover crinkle from the Kerr's Pink at this stage nothing but a mild mosaic was obtained in President in both experiments. The final observations on the two stocks of Kerr's Pink were that within two to three years after infection there was no further visible evidence of crinkle, but a mild mosaic was generally apparent.

These experiments appear to establish the fact that Kerr's Pink is capable of being infected with crinkle, but afterwards throws off part of the complex. The recovery is not due to the dying out of diseased plants, but to the actual recovery of plants which were once infected, and in which the disease must have been systemic, since it affected the tubers. This occurrence is unusual in the case of virus diseases, but it is not so rare perhaps as has been supposed, for it is not entirely confined to this particular potato virus. The fact that the inoculated Kerr's Pink plants eventually conveyed a simple mosaic alone to President goes to prove that this disease enters into the crinkle complex. The experiment, indeed, may be interpreted as an analysis of this disease, for Kerr's Pink provides a means by which the A constituent may be filtered out, leaving the mosaic constituent behind. Incidentally, the same variety has been found to filter streak out of a combination of streak and interveinal mosaic, but how regularly this occurs is not known. These results are very interesting as showing that similar filtering effects can be secured with certain potato varieties as K. M. Smith (18) obtained with *Datura* and *Petunia,* without the danger of complications which sometimes appear to follow from the passage of potato viruses through other plants (7).

Observation of cases of recovery from crinkle and streak has led us to suppose that violent necrosis of the foliage is sometimes a prelude to the disease being thrown off, either completely or in part, that is, if the plant survives. It has also been observed, in the case of diseases in which the tubers are capable of being similarly affected, that if the circumstances are such as to permit necrosis to develop on the foliage, the tubers are more likely to be unblemished. The explanation of these phenomena remains to be discovered, but it is tempting to consider the view that, if the virus is localized in these cases in the visible lesions (1), the complete death of the tissues involved is connected with the ultimate disappearance of the disease. In the case of Kerr's Pink, necrosis and partial recovery from crinkle are certainly associated, but this is not invariable. A similar partial recovery from this disease has also been observed once in Arran Comrade, on which the symptoms are those of a severe mosaic without appreciable black spotting.

Recovery from a virus disease has been recorded by Johnson (2) in the case of true tobacco mosaic in potato and in *Nicotiana glutinosa* (3); by Storey (following Kunkel (4)) in the case of streak of maize in sugar cane (19); by Kunkel (4) in the case of grass mosaic in sugar cane; and by Valleau and Johnson (20) in the case of true tobacco mosaic in potato. All these cases have a number of points in common, namely, that the virus appears to be naturally foreign to the plant which recovers, and that recovery is associated with necrotic

lesions. The latter feature is least marked in the disease which has been so fully investigated by Storey. The degree to which the infection becomes systemic probably varies in individual instances. The case of crinkle in Kerr's Pink appears to be peculiar to a certain extent, because this virus is natural to potatoes, and because the disease appears to become fully systemic at first, since it reaches the tubers.

Production of crinkle in healthy President by combination of virus A from Irish Chieftain with simple mosaic from Arran Victory.

The next step was to see if the principle present in Irish Chieftain could be transferred to a virus-free plant of another variety, in which it would be expected that crinkle would follow on the introduction of simple mosaic.

The first attempt in this direction was made in 1929. A scion from Irish Chieftain carrying virus A was grafted on a healthy President plant on May 16th, and on July 1st this plant was divided. On the latter date one of the resulting portions was grafted with simple mosaic from Arran Victory, while the other portion was not grafted. Twenty days later crinkle-like symptoms began to develop on the double-grafted plant in the immediate vicinity of the second lot of scions, and as time went on the full symptoms of crinkle appeared. The control portion, which was grafted with Irish Chieftain alone, was practically healthy in appearance at this time, but considerably later in the season a severe mosaic developed on it also. In the sequel, both plants showed crinkle-like symptoms in the following year.

The explanation of this exceptional result[2] is to be sought for in uncontrolled infection of the control plant with simple mosaic late in the previous year, and a repetition of the experiment was clearly necessary. This was done in 1930 in duplicate, as follows:—

A control core, carrying an eye or bud, was taken from a healthy President tuber, and a plant was grown from it which received no further treatment. In the meantime the remainder of the tuber was core-grafted with Irish Chieftain which carried virus A. Two plants were raised from the grafted portion, and, to be doubly assured, both were top-grafted with the same Irish Chieftain. At a later date one of them was further top-grafted with simple mosaic from Arran Victory. Three President plants were thus present (derived from portions of one originally healthy tuber), the treatment and symptoms of which are shown in Table III.

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[2] A similar inoculation has been made at least seven times in the course of other work, but in no other case did crinkle develop.

TABLE III.—Combination of disease A and simple mosaic in President.

Particulars of three President plants derived from same tuber.		Treatment of plants.	Results first year.	Results second year.
0·45	Healthy President	Control core—no treatment	Healthy	Seven plants, all healthy
0·73	Healthy President	+ grafts of Irish Chieftain carrying virus A	Faint yellowish mosaic, like simple mosaic	Five plants, all showing faint yellowish mosaic as in first year
0·131	Healthy President	+ grafts of same Irish Chieftain; + grafts of simple mosaic later	First, faint yellowish mosaic, as above; followed by crinkle symptoms 19 days after second grafts, on shoots nearest mosaic scions	Four plants, of which three showed a crinkle-like disease and one the same faint mosaic as above

The duplicate experiment was carried out at the same time in a similar way, and the result was confirmatory. Grafting healthy President with Irish Chieftain produced a diffuse more or less clear yellowish mosaic, with flat leaves, something like simple mosaic, and the same disease reappeared in the following year. The addition of a graft of simple mosaic to this intensified the symptoms, producing small curled leaves; and when tubers from this plant were grown, four of them produced plants indistinguishable from crinkle, and one a plant showing the faint diffuse yellowish mosaic, exactly as in the previous experiment. The control plant was healthy in the first year, as were also its six representatives in the following season.

Comparison of artificially produced crinkle with normal crinkle.

It may, therefore, be taken as established that the combination of simple mosaic with the virus present in Irish Chieftain produces a disease indistinguishable from crinkle. The question arises next—is the synthetic disease really crinkle, or merely something closely resembling or approximating to it? The evidence that has been accumulated up to the present points to the two diseases being identical not only in appearance, but in their underlying qualities.

So far as the appearance goes, the symptoms of crinkle on President are striking and individual. The synthetic disease has been grown beside it on a number of occasions during four seasons both under glass and in the open, and while at times it was thought that the combination resulted in somewhat larger plants, with the leaves ridged rather than crinkled, showing less marginal ruffling, and sometimes with a tendency for the mottling to develop around the margins, at other times no distinction whatever could be established. The intensities of the symptoms produced by crinkle are, of course, somewhat variable,

as in the case of other virus diseases, depending on prevalent conditions, and similar variations are to be expected in the combination. On the whole, it may be said that the two diseases appear identical, and that it would be impossible to tell one from the other. (Compare Figs. 5 and 6.)

The symptoms of the two diseases have also been closely observed in Irish Chieftain over a period of five years. The appearance of crinkle in this variety is striking, and is very familiar, because our stock of the disease originated in Irish Chieftain, and has been largely perpetuated in it since. Throughout these five years numerous examples of both diseases have been grown under glass and in the open, and the general conclusion throughout has been that they are identical.

The symptoms of crinkle on President and Irish Chieftain are very similar, and may be said to represent the normal form, or at least the best known form, of the disease. On certain other varieties, however, a strikingly different appearance is presented. Thus, on Up-to-Date (among other sorts) the effect is very severe, and indeed usually fatal. Following infection, dark necrotic spots appear on the leaves, with streaking of the veins and petioles. The leaves are sometimes killed, and remain hanging, or the upper portion of the shoot may be killed. If tubers are produced there are lesions on their surfaces, and the plants they give rise to (if any) are usually necrotic dwarfs. This result has been secured from crinkle inoculations into Up-to-Date in five experiments in different years from 1924 to 1930, and crinkle of Irish and Dutch origins has produced the same effect (Fig. 12).

In determining, therefore, whether the synthetic disease now being treated of was really identical with crinkle, the reaction to it of Up-to-Date and similar varieties was considered decisive. Although only a small number of trials have been made in this connection, necrotic spotting of the leaves, streaking, dropping of the leaves, and in some cases complete death of the plant, have been brought about in Up-to-Date by inoculation with a combination of simple mosaic and the A disease. In one case the two diseases were combined by infecting Irish Chieftain with simple mosaic, and in the other by combining disease A from Irish Chieftain with simple mosaic in President. The effect of both combinations on Up-to-Date was the same.

Combination of interveinal mosaic with disease A from Irish Chieftain.

As may be seen by reference to Table I, the combination of interveinal mosaic and streak, respectively, with virus A also produced a crinkle-like disease in Irish Chieftain. It is necessary to pursue this matter somewhat further, although little attention has been paid to these combinations in comparison with that of simple mosaic and the A disease.

So far as interveinal mosaic is concerned, it may be said at once that a combination of it with disease A in Irish Chieftain would probably pass for crinkle in the field, particularly in the early stages of growth. The com-

bination has been under observation during the seasons 1927, 1928, 1929, and 1930, and it has been noticed throughout that it has a somewhat less serious effect on vigour than the combination of simple mosaic and virus A, or crinkle itself. The plants present a pronounced crinkle-like appearance in the early stages of growth, but later the leaves become larger, flatter, and of a better green colour, with less crinkling, ruffling, and curling, and fewer or no marginal indentations. Mottling is clear, but not markedly interveinal. As the season advances the interveinal mosaic combination suggests interveinal mosaic rather than crinkle, and the plants generally have a more normal appearance than in the case of the latter disease.

The combination of interveinal mosaic and the A disease has been conveyed from Irish Chieftain to President on three occasions in different seasons, with striking results, which usually differ in a radical manner from those of the corresponding simple mosaic combination. In the first experiment (1928) the three President plants inoculated by means of core-grafts developed severe necrotic spotting and death of the leaves, along with incipient symptoms of interveinal mosaic. In the second experiment in 1929, the same combination in Irish Chieftain was fatal to three President tubers into which it was grafted, with the result that no plants were produced. Interveinal mosaic from six other varieties was similarly grafted into President tubers at the same time, all the tubers being grown under the same conditions, but in no other case did the disease prove fatal to President. The reason is, presumably, that only in the case of the interveinal mosaic from Irish Chieftain was another virus present in addition. In the third transfer to three President plants in 1930, again by core-grafts, there was again some necrotic spotting and streaking of the leaves, but eventually the plants recovered from this, and two of them came to resemble typical crinkle and one interveinal mosaic.

The initial effects of interveinal mosaic and disease A on President are unexpectedly severe, seeing that neither disease alone is very serious, the latter being least so. It is to be noted that the stock of interveinal mosaic used has always produced necrotic discolourations in President tubers. It is possible that this necrosis is due to the admixture of another virus, in which case it remains to be seen what effect pure interveinal mosaic, in combination with the virus A, would have on the variety President.

Coupled with the more variable and more severe symptoms, the presence of necrosis helps to differentiate the combination of interveinal mosaic with virus A from natural crinkle on President, although some tuber necrosis occasionally accompanies the latter disease also. No doubt the differentiation would be possible under experimental conditions, but in the field this is doubtful, and it would perhaps be impossible in the case of a number of other varieties. With the information at present available it is probably best to look on this combination as a form of crinkle, not at all times readily distinguishable by its appearance. The probable occurrence of varieties of this disease is discussed later.

Combination of forms of streak in Up-to-Date with disease A in Irish Chieftain.

So far as Up-to-Date streak is concerned, notwithstanding the results shown in Table I, considerable caution, to say the least, is necessary before it can be concluded that a combination of streak and the A disease produces crinkle, and we are of opinion that this is not the case. The reason for this conclusion is twofold. In the first place, the streak-crinkle complex produced by a combination of the viruses present in streak Up-to-Date with that present in Irish Chieftain is readily distinguishable from crinkle in President, although the two diseases are similar in Irish Chieftain (Table IV). This is because President shows up the streak constituent, to which Irish Chieftain is largely tolerant. In the second place, evidence will be presented to show that the streak virus in Up-to-Date is accompanied by a simple mosaic, and that it is the latter, and not streak, which combines with virus A to give crinkle. The results, as will be seen, are in full agreement with our previous conclusions concerning the constitution of the latter disease.

To deal first with the presence of a simple mosaic along with streak, it may be said that the second, third, and fourth stocks of streak which were used in these experiments were being tried in an endeavour to replace an original stock, which was believed to have been mosaic-free. The presence of mosaic in these three stocks has been repeatedly shown by the appearance of mottling on recently inoculated plants before necrotic spots appear, and also by the reappearance of mosaic in plants which recover from streak, as even such susceptible varieties as President and Arran Victory sometimes do. Mosaic has also been clearly seen on, and in some cases recovered from, such streak-tolerant varieties as Arran Chief, Great Scot, and Arran Consul, following inoculation with one or other of these streak stocks. The presence of simple mosaic would, as has been shown, lead to the production of crinkle, on combination with virus A, even in the absence of streak. On the other hand, the presence of streak in the complex is revealed when the latter is transferred to President, on which the usual symptoms of streak, as well as those of crinkle, are produced. These two diseases are easily differentiated on this and other similar varieties.

The most decisive proof, however, that streak is not an essential factor in the crinkle produced when the Up-to-Date and Irish Chieftain viruses in question are combined, is furnished by the fact that streak-free Up-to-Date is capable of producing a similar crinkle under the same conditions. The occurrence of an Up-to-Date plant which is unaffected by streak is somewhat of a rarity, and when after many years an example was found in 1927, the absence of this disease was twice proved in successive years by grafting on the streak-intolerant varieties President and Arran Victory. There was no evidence whatever of streak, but the presence of simple mosaic was established in the Up-to-Date in these experiments (Figs. 7 and 8). There were thus available for comparison

Up-to-Date carrying streak and a simple mosaic, and Up-to-Date carrying a simple mosaic alone. The effect of inoculating these viruses into Irish Chieftain carrying virus A is shown in Table IV.

TABLE IV.—Combinations of streak-carrying and streak-free Up-to-Date with Irish Chieftain carrying disease A.

Inoculated plant.	Grafted with	Effect on Irish Chieftain.		Recovery from Irish Chieftain on President.	
		1st year.	2nd year.	Effected first year by top-graft.	Effected second year by core-graft.
Irish Chieftain 9.225	Up-to-Date carrying streak and a simple mosaic	Pronounced crinkle symptoms at first, later moderating	Like mild form of crinkle	Mottle followed by streak. Tuber lesions [1]	2 plants streak; 1 plant crinkle-like, moderating
Irish Chieftain 9.229	do.	do.	do.	—	1 plant streak; 1 plant crinkle-like
Irish Chieftain 9.222	Streak-free Up-to-Date (carrying a simple mosaic)	Pronounced crinkle symptoms	do.	Like mild form of crinkle [2]	3 plants, all like mild crinkle
Irish Chieftain 9.224	do.	do.	do.	—	3 plants, all like mild crinkle

[1] Streak re-appeared in following year, killing seven plants out of nine, and causing two to be streak dwarfs.

[2] The same mild crinkle-like disease re-appeared in following year.

The interpretation of these results is simple—for both of the complexes (streak + a simple mosaic + A disease) and (a simple mosaic + A disease) produced the same external crinkle-like symptoms in Irish Chieftain. In the second season no difference whatever could be seen between the two combinations (Table IV, col. 4). Evidently, therefore, the presence of streak was not essential for the production of crinkle symptoms, though it made no difference in the result, as far as appearances in Irish Chieftain are concerned. This was to have been expected, since it was known that this variety is tolerant of streak. The recovery on President, however, showed that there was a difference between the crinkle-like disease deriving from the streak-carrying Up-to-Date and that from the streak-free Up-to-Date. When streak was absent from the combination, the disease was recovered on President as a recognisable crinkle, but when streak was present, it showed itself obviously on most of the President plants (Table IV, last two columns). See Figs. 9, 10, and 11.

The evidence available from other sources is also entirely corroborative. In two series of experiments carried out in 1928, 1929, and 1930, all efforts to

produce crinkle by combining Up-to-Date streak with other mosaics (virus A being absent) ended in failure. The combination of streak with simple mosaic in President and Arran Comrade produced symptoms of both diseases, but no trace of crinkle. A natural combination of Up-to-Date streak and interveinal mosaic has also been worked with, and this never showed any affinity to crinkle. Furthermore, the addition of streak to the somewhat mild and variable crinkle produced by combining interveinal mosaic with the A disease did not intensify the crinkle symptoms in Irish Chieftain or President. Again, the addition of streak to normal crinkle in Irish Chieftain did not alter or accentuate the original symptoms, but the presence of an impurity became evident in further inoculations from this source, carried out on the usual test varieties.

It is clear, therefore, that Up-to-Date streak has no essential part in the composition of crinkle. It may exist in the case of certain varieties side by side with crinkle without altering the appearance of the latter, but in this case it is a latent superfluity. If an analysis of such a "crinkle" is made, streak will appear as one of its constituents, but it has no part in the production of the characteristic symptoms. The superfluous nature of streak in the combination is revealed when this disease is transferred to President, which is a good differential variety, for the plants will be found to suffer severely from the streak, and the development of the usual crinkle symptoms will be largely prevented. The recent conclusion of K. M. Smith (18) that there is a non-variable necrotic virus (virus Y) common to these two diseases (as well as to others), is not in harmony with our results. This matter is more fully discussed by one of us in another paper (7).

Varieties of crinkle produced by the combination of various mosaics with the A virus.

It is necessary to state that the synthetic disease produced, as has just been described, by combining a simple mosaic in Up-to-Date with the A disease in Irish Chieftain, while it was more like crinkle than any other known disease, was not so severe in its effects on the foliage as standard crinkle. Similarly, it was not so severe as the crinkle disease artificially compounded of our standard simple mosaic and virus A. The same lack of full intensity characterised this disease when it was recovered from Irish Chieftain in President in two separate experiments extending over two years. We have been accustomed to describe it as "a mild form of crinkle" or "sub-crinkle" (Tables II and IV), and have found that its character is permanently fixed. The symptoms presented are, generally speaking, those of full crinkle early in the season, including very clear mottle, crinkling, ruffling, stoppage of growth at points along the leaf margin, and moderate dwarfing. Later on, the plants make a notable recovery. The symptoms of mosaic are more or less retained on the lower leaves, but the upper portion of the plant becomes almost normal, although some of the terminal growths may show evidence of more pronounced disease again later. The same

cycle of changes is repeated in the following season, and both President and Irish Chieftain react similarly to the complex. During the latter portion of the season the disease sometimes suggests interveinal mosaic, or rather this disease after it has been passed through Irish Chieftain. (Compare Fig. 10 with Figs. 3 and 4.)

The combination of interveinal mosaic with A disease from Irish Chieftain is also pleomorphic in character, as has been pointed out, and shows a similar well-marked recovery as the season advances. This may indicate that the mosaic present in the Up-to-Date used has affinities with interveinal rather than simple mosaic. The matter has not been further pursued, but it has been noticed that the Up-to-Date mosaic has a less severe effect on President[3] than our stock simple mosaic.

It is probable that "crinkles" corresponding to all these artificial combinations occur in nature. At some stages of growth they might not be recognised as such, but at others they would all probably pass for crinkle. It is likely that each variety of simple or interveinal mosaic, for the existence of which some evidence is available, can enter into combinations, with virus A for instance, and thus produce varieties of the more severe virus complexes. Among the varieties of crinkle produced, the combination of standard simple mosaic from Arran Victory with disease A in Irish Chieftain corresponded most closely with standard crinkle as it occurs naturally in Irish Chieftain and other varieties.

Some Characteristics of the A disease.

It has been assumed up to the present that the principle naturally present in Irish Chieftain is a virus. On the whole, its behaviour and transmission correspond to those of viruses, and there is no other category known in which it can be placed. In certain respects, however, it appears to differ from most other viruses of the potato, notably in being less completely systemic in the plant, resulting in recovery in certain cases, and in its more restricted host range. Parallels can, however, be found among the virus diseases of other plants, and even in the potato disease known as streak.

Irish Chieftain containing virus A usually presents few or no symptoms, as has already been stated. A faint mosaic mottle accompanied by a slight marginal undulation is frequently seen in the early stages of growth under glass, or at least the plants are always suspicious at this time, but later they become more normal, and no definite symptoms of disease can be observed (Fig. 1).

On the variety President the symptoms are a very faint mosaic, without any distortion of the leaf. Following infection, a slight general chlorosis appears on the upper portion of the plant, and on close examination a yellowish mottling can be seen, which consists of partially green areas interspersed with normally green ones (Fig. 2). Arran Victory has been inoculated twice with the

[3] This experiment shows, incidentally, that crinkle was not latent in the Up-to-Date used, but was only produced when the Up-to-Date virus was passed through Irish Chieftain.

disease, and in each case a slight mosaic followed, either in the same season or the following one. Arran Victory inoculated with paracrinkle at the same time developed the usual well-marked symptoms of that disease. The A disease is not easily demonstrable on any of these varieties, but the characteristic symptoms of crinkle are produced on the addition of simple mosaic. It will probably be found that virus A produces a somewhat similar slight effect on all varieties on which crinkle assumes its normal appearance of a severe mosaic, with little or no accompanying necrosis.

An entirely different picture is presented when the virus is inoculated into Up-to-Date, which, however, contains a simple mosaic, no virus-free stock being available. In effect, therefore, the symptoms produced should be referred to crinkle, since this would be expected to result from the combination of these two viruses. The addition of the A virus to this Up-to-Date results in a severe necrotic disease, which is often fatal. The symptoms are those usually associated with streak, consisting of black-spotting of the lamina of the leaf, streaking of the veins, petioles, and stems, leaf-drop, dwarfing, and premature death. Necrosis appears also in some of the tubers, sometimes with fatal effects. In severe cases, circular lesions appear in autumn on the tuber surface, beginning as an annular area and then becoming a closed circle. The overlying skin is at first translucent, and later it becomes dark, and cracks open. On cutting, such tubers are found to be affected throughout, showing in the flesh circles of brown discoloured tissue surrounding very necrotic centres. The effect on the tubers is very variable, as in other similar cases, and the disease appears at times to produce no visible injury. It is extremely interesting to note that crinkle itself has an exactly similar effect on Up-to-Date. The reaction of virus-free Up-to-Date, if and when it becomes available, still remains to be determined. It is anticipated that it will be found similar to that of British Queen and other varieties which fall into the same class (Fig. 12).

Work in this direction is still in progress, but it is intended to show in the near future that potato varieties can be divided into groups which have similar reactions to the commonly occurring virus diseases. Among other things, it will be shown that each of these diseases, including simple mosaic, produces serious necrosis on at least one group of varieties, but the groups vary with the disease in question. This fact, therefore, does not indicate the possession by all the diseases of a common and invariable necrotic element (18), but rather of different or variable necrotic elements. In view of the extent to which necrosis accompanies both crinkle and streak it would be a natural but, as (it is believed) the evidence clearly shows, an incorrect view to look upon them as of similar composition. It has already been shown that crinkle cannot be compounded with Up-to-Date streak as a constituent, and it is also clear from the effect on Up-to-Date of virus A that the latter is entirely distinct from the Up-to-Date streak virus. It is not improbable, perhaps, that virus A has affinities with the streak group, but it is certainly not the streak virus which occurs in Up-to-Date.

The A disease is, furthermore, not identical with any other potato virosis known to the present authors, and for this reason they have given it a provisional name, until its nature and status can be more exactly determined. In repeated trials no symptoms have been produced by it on tobacco or *Datura Stramonium,* and there is some evidence to show that infection, if it ever takes place, never becomes systemic in the latter.[4] Under the same conditions infection was readily secured from other potato mosaics in those plants. The failure to produce visible symptoms in tobacco readily differentiates the virus from most other viruses of the potato, or at least from those which have been adequately described. The question of the host range is still being studied, but up to the present no plant, other than certain potato varieties, has been found on which the virus produces a characteristic reaction.

Virus A is widespread, and indeed according to our experience universal, in plants of Irish Chieftain in nature, and will probably be found to occur in other varieties. This matter has not been particularly investigated, but the next most likely place to look for it is in Golden Wonder. Both simple and interveinal mosaic produce a crinkle-like disease on this variety, and a somewhat similar disease is recovered from it on President. Certain commercial seedlings which have been examined show similar characteristics.

Conclusion.

The facts recorded in the present paper are of considerable practical interest. The discovery of virus A, and the proof that the combination of this with a number of mild mosaics produces crinkle, or crinkle-like diseases, is a step towards the elucidation of the origin and occurrence of the more severe forms of mosaic. The growing of varieties in which this virus naturally occurs is likely to lead to the development of crinkle in other varieties. Nothing is yet known as to the agency by which this disease is propagated in the field. The partial transmission which has been recorded (17) may find its explanation in the results recorded in this paper. In any future work in this connection the fact that crinkle is a complex must be borne in mind, although some of our field observations have been interpreted as indicating that it may be transmitted in nature as a unit.

The discovery of the part played by virus A when it combines with simple mosaic is important in another way. The former is carried by certain varieties without any marked symptoms being visible, but a very severe mosaic (*i.e.,* crinkle) results when simple mosaic is introduced in addition. This does not represent the effect of simple mosaic on the variety, but that of the combination. In testing new varieties for their reaction to virus diseases, and in other similar work, it is necessary to use virus-free stocks, as far as available, and the absence of virus A must now be established, in addition to that of other possible latent viruses. The usual test varieties, President and Arran

[4] We are indebted to our colleague, Dr. Clinch, for some notes on this question.

Victory, are not particularly suitable for this purpose, unless supplemented by such a variety as Up-to-Date, or any variety affected with simple mosaic.

The more general question of the bearing of the results here recorded on other work concerning the occurrence of virus complexes in the potato is discussed by one of us in another paper (7), to which the reader is referred.

SUMMARY.

An experiment, repeated three times at different periods, in which simple mosaic was introduced into Irish Chieftain carrying another virus, designated A, resulted in a disease which was indistinguishable from crinkle, and which persisted as such.

This crinkle-like disease, when transferred to President, again reproduced all the characteristics of crinkle in the majority of cases. In others, a mild mosaic alone resulted, due to the dropping of one of the constituents in transmission. Exceptionally, a disease intermediate between crinkle and simple mosaic was produced, the interpretation of which is discussed.

A constituent is also dropped sometimes in the graft-transmission of naturally-occurring crinkle. The variety Kerr's Pink, amongst others, is capable of being systemically infected with this disease, but afterwards recovers, filtering out one constituent and leaving behind a mild mosaic.

When, in two separate experiments, virus A was introduced into healthy President, and following this simple mosaic was inoculated into the same plant, typical and persistent crinkle symptoms again developed in most cases, and a faint mosaic in others.

The synthetic crinkle produced in the two ways described above caused the same typical necrotic disease as naturally-occurring crinkle in Up-to-Date.

A combination of interveinal mosaic and virus A produced an aberrant form of crinkle, which may be considered as a variety of this disease.

It is shown that the production of a crinkle-like disease when streak from Up-to-Date was combined with virus A was due to a simple mosaic occurring as an impurity along with the streak. This streak has no part in the constitution of crinkle.

The probable occurrence of varieties of crinkle is pointed out.

Some of the characteristics of the A disease are described, and its practical importance is discussed.

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EXPLANATION OF PLATES.

PLATE 10.

Fig.

1. Leaf of plant of Irish Chieftain affected with virus A. There are normally no symptoms of disease on fully-grown plants of this variety.
2. Virus A in President, showing a slight general chlorosis or mottle.
3. Naturally occurring crinkle in Irish Chieftain.
4. Synthetic crinkle produced by infecting with simple mosaic Irish Chieftain which carried virus A.

PLATE 11.

5. Recovery of natural crinkle in President from Irish Chieftain (*Fig.* 3).
6. Recovery of synthetic crinkle in President from Irish Chieftain (*Fig.* 4).
7. Early symptoms of Up-to-Date streak in President. Note the netted spots, which are pale green, or yellow, areas in which the veins become necrotic, General necrosis and death usually follow.
8. President grafted with streak-free Up-to-Date showing absence of streak symptoms. A faint mosaic is present, however.

PLATE 12.

9. Irish Chieftain grafted with Up-to-Date streak, and showing a mild crinkle-like disease. Irish Chieftain carries this streak without symptoms, and it is a simple mosaic present with the streak in Up-to-Date which combines with virus A, naturally present in the Irish Chieftain, to give mild crinkle. Compare with *Fig.* 10. For recovery on President see *Fig.* 11.
10. Irish Chieftain grafted with Up-to-Date which is streak-free, but contains a mild mosaic. A mild form of crinkle follows, as in the last case, due to the combination of mild mosaic in Up-to-Date with virus A in Irish Chieftain. Compare with *Fig.* 9.
11. Recovery on President of form of crinkle with streak complication in Irish Chieftain, as shown in *Fig.* 9. The streak, which is latent in the Irish Chieftain, becomes very obvious when the disease is transferred to President.
12. Effect of natural crinkle on Up-to-Date following a core-graft. Necrosis, accompanied by distortion, is just setting in, and usually ends fatally.

FIG. 1.

FIG. 2.

FIG. 3.

FIG. 4.

FIG. 5.

FIG. 6.

FIG. 7.

FIG. 8.

FIG. 9.

FIG. 10.

FIG. 11.

FIG. 12.

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